TRANSACTIONS AND PROCEEDINGS

OF THE

BOTANICAL SOCIETY OF EDINBURGH.

VOLUME XXX.

PART I.

SESSION 1927–28.

EDINBURGH:
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1928.

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PROCEEDINGS

OF THE

BOTANICAL SOCIETY OF EDINBURGH.

SESSION XCII

OCTOBER 20, 1927.

Professor W. Wright Smith, M.A., F.L.S., F.R.S.E.,
Vice-President, in the Chair.

The following Office-Bearers were elected for Session 1927–1928:—

PRESIDENT.

John Sutherland, C.B.E., LL.D.

VICE-PRESIDENTS.

T. Cuthbert Day, F.I.C.
W. Edgar Evans, B.Sc., F.R.S.E.
Symington Grieve, Esq.
J. Rutherford Hill, Ph.C.

COUNCILLORS.

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Professor Montagu Drummond, M.A., F.L.S., F.R.S.E.
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Andrew Harley, Esq.
J. M. Murray, B.Sc.
Miss E. Philip Smith, B.A., Ph.D., F.L.S.
W. G. Smith, B.Sc., Ph.D.
Professor W. Wright Smith, M.A., F.L.S., F.R.S.E.
Malcolm Wilson, D.Sc., F.L.S., F.R.S.E.

Honorary Secretary—J. R. Matthews, M.A., F.L.S., F.R.S.E.
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Treasurer—Andrew Mason, Esq., c/o Richard Brown & Co., C.A.
Assistant-Secretary—J. T. Johnstone, M.A., B.Sc.
Artist—R. M. Adam.
Auditor—Robert C. Millar, C.A.

Mrs. Murray Reid was elected a Resident Fellow.

Mr. George Forrest was elected an Associate.

Miss J. J. Stevenson and Miss C. M. Baron were elected Ordinary Members.

Mr. E. M. Buchanan read a paper on Types of Forest and of People in Burma. He showed how the different types of forest are related to the geographical features and geological formations of the country, and illustrated his paper with a large number of lantern slides. He also gave some account of the different types of people among the inhabitants.

NOVEMBER 17, 1927.

W. Edgar Evans, B.Sc., F.R.S.E., Vice-President, in the Chair.

The Treasurer, Mr. Andrew Mason, submitted the following Statement of Accounts for Session 1926–1927 :—

Income.

Annual Subscriptions for 1926–1927	£45 15 0
Do. Arrears	1 15 0
Transfer from Life Members' Fund	21 18 10
Transactions sold	7 7 6
Interest on Funds Invested and in Bank	13 2 8
Subscriptions to Publications Fund	24 2 0
Income from Botanical Society Trust Fund	17 14 3
	£131 15 3

Expenditure.

Printing *Transactions* for Session 1925–1926	£70 15 5
Printing and Postage of Notices for Meetings, etc.	21 2 7
Rooms for Meetings and Tea	7 3 10
Stationery, Postages, Advertising, etc.	4 7 10
Fire Insurance on Books, etc.	0 5 0
Honorarium to Treasurer	3 3 0
	£106 17 8
Excess of Income over Expenditure	£24 17 7

State of Funds.

Life Members' Fund.

Balance of Fund at close of Session 1925–1926	£263 15 10
Add—Life compositions received	36 15 0
	£300 10 10
Deduct—Transferred to Income	21 18 10
Balance as at close of Session	£278 12 0

Brought forward,		£278 12 0
Ordinary Fund.		
Balance of Fund at close of Session 1925–1926	£95 9 6	
Add—Increase during Session 1926–1927	24 17 7	
Balance as at close of Session, subject to expense of printing *Transactions*		120 7 1
Total Funds . .		£398 19 1
Being :—£200 5% War Stock, 1929–1947, at cost	£194 18 3	
Sum in Current Account with Union Bank of Scotland, Ltd. .	5 0 10	
Sum in Deposit Receipt with do.	200 0 0	
	£399 19 1	
Less—Subscription received in advance	1 0 0	
As above .		£398 19 1

Note.—Subscriptions in arrear, considered recoverable: 1925–26, 15s.; 1926–27, £5, 10s.

EDINBURGH, *7th November* 1927.—I hereby certify that I have audited the Accounts of the Treasurer of the Botanical Society of Edinburgh for Session 1926–1927, and have found them correct. I have also checked the foregoing Abstract, and find it correct.

ROBT. C. MILLAR, C.A., *Auditor.*

BOTANICAL SOCIETY TRUST FUND.

INCOME.

Interest on Funds invested £17 14 3

EXPENDITURE.

To Publications Fund £17 14 3

EDINBURGH, *7th October* 1927.—I have examined the books and vouchers of the Edinburgh Botanical Society Trust Fund, and certify the same to be correct.

ROBERT L. GORRIE.

Miss MARGARET GAIRNS DOBSON and Mr. J. M. S. LANG were elected Resident Fellows.

Mr. ALEX. B. BROWN, Mrs. BUYERS, Miss EVANS, Miss ELSA DRYDEN ROBERTSON, and Mr. JAMES WRIGHT were elected Ordinary Members.

Mr. R. M. ADAM read a paper on *Codium adhaerens* Ag. and other Algae from West Inverness-shire. Hitherto *Codium adhaerens* Ag. has not been reported from any Scottish locality and its discovery in Loch-nan-Uamh adds considerably to the

geographical range of the species. It has already been found in a few localities in western Ireland and the shores of southern England. The paper was illustrated with specimens and lantern slides.

Mr. Arthur Bennett communicated a paper: Notes on Caithness Plants (see p. 1).

Mr. J. Rutherford Hill exhibited a fine specimen of Peloria in Foxglove.

Specimens of *Cnicus heterophyllus* var. *laciniosus* were exhibited on behalf of Mr. Arthur Bennett.

Mr. L. B. Stewart exhibited specimens of *Zamioculcas Loddigesii* showing regeneration from leaflets.

The following plants in flower were shown from the Royal Botanic Garden :—*Aphelandra aurantiaca* Lindl. var. *Roezlii*; *Callicarpa purpurea* Juss.; *Casuarina distyla* Vent.; *C. quadrivalvis* Labill.; *Clematis ranunculoides* Franch.; *Columnea gloriosa* Sprague; *Cymbidium erythrostylum* Rolfe; *C. Mastersii* Griff.; *Dendrobium Victoriae-Reginae* Loher; *Dichorisandra thyrsiflora* Mikan; *Hoheria populnea* A. Cunn.; *Masdevallia laucheana* Kraenzl.; *Pinguicula caudata* Schlecht., and *Polystachya puberula* Lindl.

DECEMBER 15, 1927.

John Sutherland, C.B.E., LL.D., President, in the Chair.

Mr, E. Wyllie Fenton was elected a Resident Fellow.

Miss A. Margaret Mitchell was elected an Ordinary Member.

Mr. W. Edgar Evans moved that the following Alteration of Laws be made :—

CHAPTER IV.—Admission of Members.

That Section II., paragraph 3, be altered to read :

> Resident Fellows may at any time compound for their annual contributions by payment of Ten Guineas. They shall be entitled to receive the *Transactions* yearly as published.

This was seconded by Mr. J. Rutherford Hill and carried unanimously.

Mr. J. Rutherford Hill moved that the following Alteration of Laws be made :—

CHAPTER IV.—Admission of Members.

That Section III., which deals with Non-Resident Fellows, be deleted.

This was seconded by Mr. Symington Grieve and carried unanimously.

Miss M. J. F. Wilson read a paper on the Dutch Elm Disease, a serious disease on the Continent, and recently discovered near London. The paper was illustrated by a large number of lantern slides.

The following plants in flower were shown from the Royal Botanic Garden :—*Angraecum caespitosum* Rolfe ; *Billbergia zebrina* Lindl. ; *Cochlioda vulcanica*, Benth. ; *Coelogyne assamica* Linden et Rchb. f. ; *Cymbidium Coningsbyanam* × ; *C. elegans* Lindl. ; *C. Hanburyanum* × ; *Epidendrum ciliare* Linn. var. *giganteum* ; *Eriogonum nudum* Dougl. ; *Laelia anceps* Lindl. ; *Lilium nepalense* D. Don ; *Maxillaria grandiflora* Lindl. ; *Oncidioda Charlesworthii* × ; *Oncidium cheirophorum* Rchb. f. ; *Ornithidium coccineum* Salisb., and *Platyclinis Cobbiana* Hemsl.

JANUARY 19, 1928.

John Sutherland, C.B.E., LL.D., President, in the Chair.

Miss Mary S. Martin was elected an Ordinary Fellow.

Dr. W. G. Smith read a paper : Notes on the Effect of Cutting Bracken, and illustrated it with lantern slides and specimens (see p. 3).

Miss Mary S. Martin read a paper : A Note on Seed-borne Disease of Clover by Mrs. N. L. Alcock and herself, and illustrated it with lantern slides (see p. 13).

Miss Elsie Cadman exhibited cultures of *Didymium difforme* and *D. nigripes*.

The following plants in flower were shown from the Royal Botanic Garden :—*Ardisia mamillata* Hance ; *Chorizema ilicifolium* Labill. ; *Coelogyne sulphurea* Rchb. f. ; *Cymbidium*

edinensis ×; *Echeveria multicaulis* Rose; *Eria globifera* Rolfe; *Felicia echinata* Nees; *Masdevallia Schroederiana* Sander; *Odontoglossum Groganiae* ×; *O. Uroskinneri* Lindl.; *Senecio grandifolius* Less.; *Visnea Mocanera* Linn. f., and *Xylobium scabrilingue* Rolfe.

FEBRUARY 16, 1928.

John Sutherland, C.B.E., LL.D., President, in the Chair.

Mrs. George Forrest, Mr. John Brodie, Mr. Charles D'Arcy McGinn, Dr. John Lewis Owen, and Mr. William Henry Traill were elected Ordinary Fellows.

George Forrest, jun., John Eric Forrest, and Miss Peggy Pearson were elected Ordinary Members.

The President announced the death of Sir Dyce Duckworth, the oldest member of the Society, and also of George Muirhead, LL.D., who had been a member since 1878.

Dr. James W. Gregor read a paper: Observations on Timothy Grass and a Hybrid (*Phleum pratense* × *P. alpinum*), which he illustrated by lantern slides.

The following plants in flower were shown from the Royal Botanic Garden:—*Bulbophyllum tremulum* Wight; *Catasetum macrocarpum* Rich.; *Dendrobium delicatum* Bailey; *Geranium crithmifolium* Poir.; *Laelia Lundii* Rchb. f.; *Loropetalum chinense* Oliver; *Lycaste Skinneri* Lindl. var. *alba*; *Maxillaria variabilis* Batem. var. *lutea*; *Porphyrocoma lanceolata* Scheidw.; *Primula Winteri* W. Wats.; *Rhododendron argenteum* Hook. f.; *Rh. mucronulatum* Turcz.; *Rh. moupinense* Franch.; *Saxifraga Biasoletti* ×; *S. Burseriana* Linn. var. *gloria*; *S. Dörfleri* ×; *S. Hörhammeri* ×; *S. Kellereri* ×; *S. Sundermannii* ×; *Sedum compressum* Rose, and *Stifftia chrysantha* Mikan.

MARCH 13, 1928.

J. Rutherford Hill, Ph.C., Vice-President, in the Chair.

Mr. George Forrest gave an account of the Scenery and Flora of Yunnan, and illustrated it with a large number of lantern slides, showing many plants in their native habitat.

Professor WRIGHT SMITH showed and explained two lantern slides representing diagrammatically the world-distribution of the Genus *Primula*.

APRIL 19, 1928.

J. RUTHERFORD HILL, Ph.C., Vice-President, in the Chair.

Mr. V. E. M. DAVY read a paper on Bolting in Root Crops.

Dr. R. J. D. GRAHAM and Mr. L. B. STEWART read a paper on Injection Experiments on Trees (see p. 19).

MAY 17, 1928.

SYMINGTON GRIEVE, Esq., Vice-President, in the Chair.

Mrs. KATE SUTHERLAND was elected an Ordinary Fellow.

Col. H. H. JOHNSTON communicated a paper on Additions to the Flora of Orkney, as recorded in Watson's "Topographical Botany," Second Edition (1883), which was illustrated by a number of interesting specimens collected by himself.

Col. H. H. JOHNSTON also communicated a paper on Additions to the Flora of Shetland.

Miss ELIZABETH W. MILLER read a paper on the Occurrence of Schizocotyly in certain Ranunculaceous Seedlings, and exhibited a number of specimens (see p. 21).

Professor WRIGHT SMITH exhibited *Primula Reidii* in flower and also cultivated plants of *Linnaea borealis* in flower.

Mrs. N. L. ALCOCK exhibited specimens of Plum Scab on twigs of Victoria Plums, from Galashiels.

JUNE 21, 1928.

J. RUTHERFORD HILL, Ph.C., Vice-President, in the Chair.

Professor BASIL WILLIAMS and Mrs. DOROTHY WILLIAMS were elected Ordinary Members.

The Chairman announced the death since the last meeting of Mr. James A. Ferguson, J.P., a Fellow of the Society.

Miss Marjorie L. R. Bovell read a paper: Note on a Circumnutation Record (see p. 46).

Miss C. I. Kean read a paper on Light Receptors in Mesembryanthemum (see p. 37).

Miss C. I. Kean also read a paper on Artificial Crystals in preserved tissue of Mesembryanthemum (see p. 43).

Mr. George Taylor exhibited some flowering plants from South Africa.

TRANSACTIONS

OF THE

BOTANICAL SOCIETY OF EDINBURGH

SESSION XCII

Notes on Caithness Plants. By Arthur Bennett, A.L.S.

(Read 17th November 1927.)

These notes comprise any additions and new localities that have come to my notice since the publication of my last notes in the Transactions of this Society, vol. xxix, p. 54 (1924), as I hope that some day a Flora of the county will be prepared.

Viola lepida Jord.—Side of Wick River. Druce in Bot. Exch. Club Rep. for 1923, p. 171.

Viola Pesneaui Lloyd.—Dunnet Links. Druce, *loc. cit.*, p. 172.

Cerastium tetrandrum Curt. var. *eglandulosum* C. E. Salm. —Salmon in Jour. Bot., lxi (1923), p. 90.

Geranium lucidum Linn.—Near Lybster, G. Lillie, sp.

Artemisia vulgaris Linn. var. *coarctata* Forselles.—Coast near Lybster, G. Lillie, sp.

Thymus britannicus Ronn.—Thurso, Grant.

Atriplex glabriuscula Edmondst. var. *pseudo-calotheca*, var. nov.

Folia ad *calotheca* accedens, fructus longus muricatus. Coast at Reiss, G. Lillie, sp.

The specimens of this species on the Caithness coast vary considerably, but most of them are far more muricate than usual.

Atriplex glabriuscula × *maritima*.—Specimens gathered near Reiss, where the two grow together, are evidently a hybrid between them. The leaves and habit resemble the latter, while the fruit is more like the former.

Carex turfosa Fries.—Banks of the river near Wick, J. Grant, sp.

Carex Goodenowii Gay var. *juncella* Fr.—Meadow by Wick River, near Wick, E. S. Marshall, sp.

Carex binervis Sm. var. *alpina* Drej.—Near Wick, J. Grant, sp.

Carex Kattegatensis Fries, in Ind. Sem. Hort. Upsala (1857).—This is one of the most interesting Caithness plants. It is very rare in Europe, being recorded only from Bohulsän, Sweden, and Christiania (Oslo), Norway. Mr. Grant of Wick writing some years ago mentions that this Carex is plentiful in the Thurso River, growing with *C. aquatilis*, and is one of the earliest species to flower, the flowers appearing about the end of March.

Notes on the Effect of Cutting Bracken (*Pteris aquilina* L.). By William G. Smith, B.Sc., Ph.D.

(Read 19th January 1928.[1])

The Bracken fern is a well-known pest for the sheep farmer and forester. It is a heritage of the earlier woodlands, left as a dominant plant on the deeper soils formerly occupied by trees. Thence it has spread in a considerable degree into grassland and into heather, suppressing these by the dense shade of its living fronds in summer and by the covering of dead fronds in winter. The dense thickets also exclude sheep, hence many acres are rendered useless for grazing. An urgent need is a cheap and effective method for its destruction or restriction. Since the underground parts are too deep in the soil to be reached by any implement except the plough, the methods used are directed towards destroying the fronds by cutting, grazing, or spraying.

Experiments on Bracken are included in the investigations on hill pasture in Boghall Glen on the Pentlands near Edinburgh, part of the farm of the Edinburgh and East of Scotland College of Agriculture. Plots were marked off on which the Bracken was cut annually at different dates, one series was begun in 1924, another in 1925. The Bracken here is a type less tall and less dense than the true woodland form, so that there is a continuous grass turf beneath it. The denser parts carry about 50 fronds per square yard, but there is variation from place to place.

The first appearance of fronds noted was 28th April 1925, 26th April 1926, and 5th May 1927, a late year. Cutting began on some plots in the last week in May, four weeks after first appearance, and was continued at intervals. The fronds on each plot were counted, and notes made on maturity, density, etc.

The general effects of cutting may be briefly summarised. The number of fronds in the second year, after one cutting, totalled about half of the original number. The plots did not appear to be much thinned, as the ground was still well covered,

[1] The notes were continued till July 1928, when the manuscript went to press.

but during the second cutting the fronds were noted as less robust and easier to cut. The result after two years' cutting was evident, as the plots were thinly covered because the fronds were smaller, but counts showed that the number of fronds cut in the third year was about the same as in the second. In the fourth year the plots were very scantily covered, but a count showed the number to be still considerable; thus where the original number was about 50 per square yard, there were still about 11 fronds per square yard at the fourth cut. The conclusion is that while three years' cutting uncovers the grass and leaves it open to grazing stock, yet it does not entirely remove the Bracken, so that if left it could return in time.

There is some difference of opinion as to best date for cutting, and this was tested. The following table gives details for one series of plots, each 93 square yards in area.

Year.	Appearance.	Plot I.		Plot II.		Plot III.		Plot IV.	
		Days.		Days.		Days.		Days.	
1925	28th April	35	2300	47	2400	57	..	65	
		65	1790						
			4090						
1926	28th April	33	830	44	1240	50	1335	63	1344
		63	1400						
			2230						
1927	5th May	31	778						
		73	1087	45	1260	53	1154	63	1000
		Oct.	670	Oct.	1600	Oct.	1134	Oct.	692
			2535		2860		2288		1692

The first appearance of fronds varied about a week during the four years of observations, and the dates of cutting are given as the number of days after the first record—that is, from about 1st June till the first week of July. In 1925, first year of cutting, the original number was ascertained on only two of the four plots, but approximately the total number was 5000 or 50 per square yard. In 1925 and 1926 the numbers do not

include fronds that developed after the date of cutting, but in 1927, in October when the fronds were brown, a count was made to ascertain the later growth. The yearly figures for Plot I, and for each plot in 1927, show that this later development of fronds is considerable, even after cutting at nine or ten weeks old. These late fronds were generally small, but on Plot II many of the later ones became tall and strong, so that they must have built up a considerable amount of reserve food for storage in the rhizomes. A comparison of the three years shows that after one year's cutting, the next crop is about half the original number. After two years' cutting the numbers fall only slightly, but there is a considerable reduction in the average size of frond.

In the fourth year, 1928, the fronds were not cut but were counted at thirty-six days and sixty-seven days. On the latter date, 6th July, the normal Bracken fronds are nearly all over a foot high, but on the plots the condition will be seen from the large number of small ones :—

1928, first fronds seen 1st May.

	Plot I.	Plot II.	Plot III.	Plot IV.	
36 days	359	578	358	217	
67 days	553	934	613	336	(less than 1 foot)
	58	112	41	18	(over 1 foot)
	611	1046	654	354	

The most evident number is that for Plot II, cut each year in the second week of June and still carrying about a fourth of its original number of fronds. This is confirmed by the appearance of the plots in July 1928, IV is distinctly thin, II has a considerable cover, and the other two are intermediate. As the second cutting of Plot I and the single cut of Plot IV were made about the same day, a better agreement was expected, but in the absence of a count for the 1925 cut for IV, it is not known whether this plot was originally less dense.

These observations support the view that early cutting or switching of the young fronds only destroys the early ones, leaving from a half to two-thirds of the normal crop of that year to develop. These grow to a large size and if not cut again will actively nourish the plant till September. Cutting about 1st July, when the fronds are about nine weeks old,

removes most of the fronds after they have used up the food reserves and before they have time to replenish the loss.[1] This means a short season for cutting to get the best results.

An experiment was begun in 1927 to test whether an application of common salt might not induce the sheep to eat Bracken. When the curled fronds were appearing above the ground in May, a plot was dressed with crushed rock salt (2 cwt. per acre). The sheep soon found the plot and there were indications of increased grazing. The top-dressing was twice repeated during May, with the result that the grass was much grazed and many Bracken fronds were seen broken off or partially bitten while still in the curled stage. Two equal areas were counted, 8th July, and gave the following numbers of fronds: Unsalted Bracken, 500 or 30 per square yard; salted, entire fronds 178, bitten or broken off 100 fresh, and others too shrivelled to count. The effect was that the number of fronds was reduced by over a half by the sheep. In 1928 the thinned plot is still recognisable, and the salting was repeated twice. The effect of salting in May, followed by a cutting in July, tried on another plot, was a considerable thinning of the plot in 1928.

The effect of sodium chlorate, a recently introduced weed killer, was tested in 1928. On one plot 2 oz. per square yard was applied as solution in water; other plots were top-dressed with dry crystals at rates varying from ½ oz. to 3 oz. per square yard. The applications were made in the middle and at the end of May on the young fronds. The result was that the fronds became dark and shrivelled, and so brittle that they snap off just below the leafy part, so that only frond stalks were left. The grass below is also destroyed. At the end of July very few fronds had appeared, a result more effective than cutting at the end of May, for a cut plot would at the end of July carry many fronds, whereas the chlorate plot is bare. The smaller dressing, ½ oz. per square yard, was found to be fairly efficient, and further tests are in progress.

An examination of the underground parts of the Bracken has been made at intervals. The most complete samples were taken in November 1927 from four places: (*a*) uncut, (*b*) cut twice for three years, (*c*) cut in first week of July for

[1] See also "The Eradication of Bracken," J. H. Milne Home (The Scottish Journal of Agriculture, ix. 123–129, 1926).

three years, (*d*) cut four years. The method was to collect all the parts of Bracken found in a hole 2 feet by 2 feet at the surface, down to about 18 inches, below which there was none.

The underground parts of the Bracken consist of two main parts, a deeper system of thicker storage rhizomes from a half to 1 inch in diameter, with lateral branches but with few fronds. Nearer the surface there is a system of thinner rhizomes closely beset with traces of petioles and with a dense covering of fine roots. The latter are referred to as frond-bearing branches, while the thicker ones are vegetative or storage rhizomes.

The amount of rhizomes may be large, and in this case the four holes yielded respectively 52, 56, 82, and 99 feet. This means that the rhizomes are closely packed up to near the surface. The lesser amounts were taken from places where the fronds were less dense.

Each rhizome and frond-bearing branch has its origin from a terminal growing-point, easily recognised as a blunt cone (fig. 1). Soon after its formation the apical cell divides and the terminal becomes a double emergence. One growing-point is larger and gives rise to a horizontal axis, the elongation of the rhizome. The other growing-point, the frond-lateral, is at first minute and lies in a slight groove on one side of the rhizome. This ultimately gives rise to a frond, and as these laterals arise alternately to right and left of the rhizome, so the fronds occur in two rows. The leafy frond of any year arises some distance (up to some inches) behind the terminal growing-point. The course of development given in the text-books was described by Hofmeister about 1850, but some deviations were found in the material from our Bracken plots. The normal sequence is: (*a*) the leafy frond of the present year, (*b*) a club-shaped bud enclosing a curled rudimentary frond, (*c*) the frond-lateral bud, (*d*) the rhizome apical bud. If only one frond were matured each year, say 1927, then the club-shaped bud contains the frond for 1928, and the lateral part of the terminal will produce the frond of 1929. Later stages of growth include elongation of the terminal growing-point, so that the successive laterals become distributed at intervals along the rhizome. Elongation is limited to the part beyond the frond of the present year, and when the club-

shaped bud has been formed there can be little elongation between it and the green frond.

The leafy frond arises from a lateral bud, and at the base of each rachis, on the proximal side, there is a growing-point that remains after the decay of the frond; a few cases have been

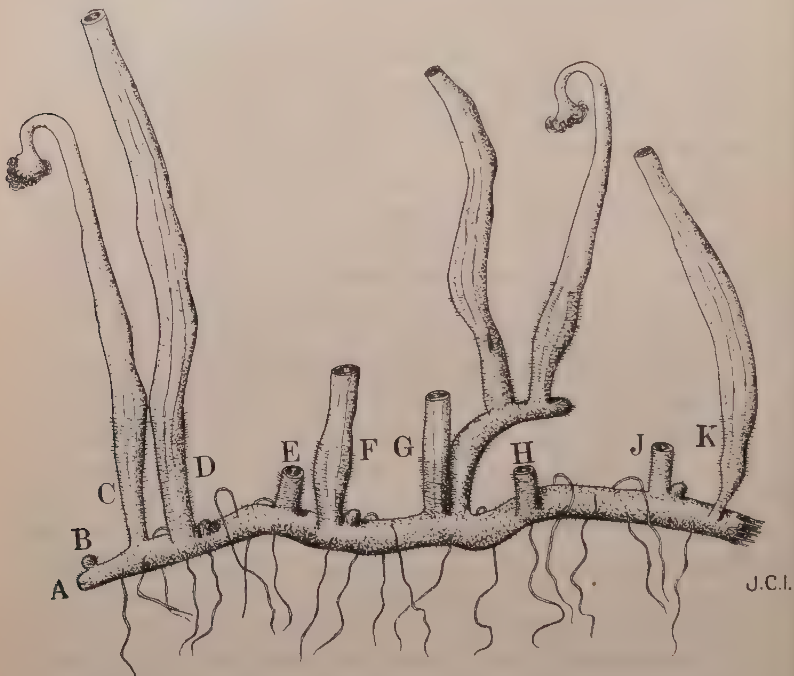

Fig. 1.—Terminal part of Bracken rhizome in May 1928; from a plant cut each year, 1925–1927.

A. Terminal growing-point. B. Frond-lateral growing-point. C. Unfolding frond of 1928. D. Petiole base 1927, with basal growing-point. E. Base of a small frond. F. Base of a larger frond with basal growing-point; both E and F probably unfolded in 1926. G. Petiole base, frond 1925; the basal bud has formed a branch with a frond unfolding in 1928. H, J, K. Petiole bases 1924, 1923, and 1922.

observed where this bud is double. This growing-point has three possibilities: (*a*) it may become a lateral branch, either vegetative or frond-bearing, (*b*) it may give rise to a second frond, (*c*) it may remain dormant (fig. 1). Dormant growing-points may be found at the base of fronds that were leafy over ten years ago, three cases were seen at fifteen years and one at eighteen. It follows that the vegetative rhizomes may be

living and vigorous up to twenty years, and capable of giving off new branches or fronds. The vegetative rhizomes do not branch freely, and most of the laterals become frond-bearing. The distance between old petiole spurs is a measure of the annual extension of the rhizome. These spurs are frequently 6 to 8 inches apart, but cases have been measured up to 20 inches. The greater elongation of the rhizome takes place in the earlier stages when the Bracken is invading suitable soil. Later, when the rhizomes become crowded, and competition for water, etc., becomes more acute, the distances are reduced.

When a frond-bearing branch arises from the deeper-seated rhizomes it inclines upwards towards the surface and apical extension is reduced, hence the petiole scars are closer, generally less than an inch. As the extremity of the branch approaches the surface it was observed that the buds and fronds became smaller, and in some cases growth had ceased. The crowding of buds was very evident in the two trial holes with 99 and 82 feet of rhizome. The effect of cutting is to increase crowding on the parts formed after cutting had begun.

When the rhizomes from trial holes were examined, depletion of the larger storage rhizomes was evident. Those from an uncut area were plump and hard for long distances and only old parts showed decay. Rhizomes from the cut plots were shrunk from a short distance behind the growing-point, and they contained a milky fluid instead of the firm white tissue of normal Bracken. Considerable lengths of the older ones had a loose cortex, frequently ruptured so that long bands of the hard sclerenchyma were exposed as broad flat fibres. No growing-points were found on these parts. The depletion extends into the bases of the frond-bearing branches, but the younger extremities may still bear growing-points. The effect of rhizome depletion is to break up what was a wide-spreading continuous plant into a number of pieces or separate plants. The fronds will be produced from tufts of branches mainly near the surface, hence the water supply from the deeper soil layers will be reduced, and the more superficial root-system must compete for water with the grasses, etc., forming the grassy turf.

This depletion of the rhizomes was more evident in the two plots, I and IV, cut for three years in July, whereas on

Plot II, cut once each year in early June, there was a greater amount of unexhausted rhizome. This has been confirmed by the investigations of J. Hendrick (Kew Bulletin, No. 4, 1921). It is there shown from two series of analyses that the amount of food reserves, as expressed by soluble carbohydrates and nitrogen, diminishes steadily from April and reaches a minimum in July, after which there is an increase.

The starvation indicated by the smallness of the fronds after two years' cutting is also seen in the buds. Each frond bears a growing-point at its base, and with each year's cutting these become smaller. The reduction in the number of fronds indicates that many of these buds no longer produce fronds, but a proportion of them can develop even after four years' cutting.

Reference to the table given will show that a considerable number of fronds are unfolded after each cutting, even when as late as July. The fronds following the earlier cuts attain to a larger size, and may be regarded as normal buds unfolding late. The later ones, however, suggest a break-away from the normal. If the rhizomes are examined in winter, the rule is to find towards the extremity of each frond-bearing branch a single club-like frond-bud elongated up to about an inch, anterior to this is a lateral knob or growing-point, and then the rhizome growing-point. Examples have been found, however, where there are two club-like frond-buds, and during summer two leafy fronds have been observed, one beside the other on the same branch (fig. 2). This has been found in various localities, so that it is certain that a branch may produce two fronds in one year. This was indicated in 1884 by Klein (Botan. Zeitung, p. 557). In this paper he questions the accuracy of Hofmeister's assumption that only two leaf rudiments exist beyond the leafy frond, and he gives illustrations showing two lateral growing-points in material collected between April and October. His sequence is: 1st year, a frond lateral (Blattanlage) in the same groove as the apical growing-point; 2nd year, an emergence (Hocker) visible to the eye and gradually leaving the groove; 3rd year, formation of the club-like bud; 4th year, unfolding of the frond. Klein suggests that two fronds may mature in one year.

The effect of cutting has been to stimulate double frond development, for on the rhizomes from our plots there is fairly

frequently, as shown in fig. 1, a thick petiole base indicating

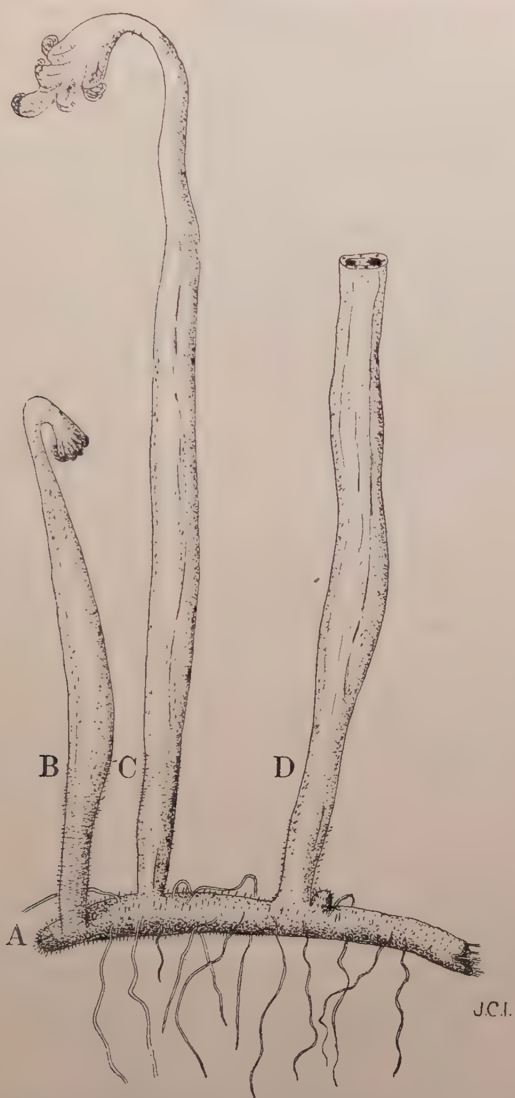

FIG. 2.—Apex of Bracken rhizome with two fronds unfolding in May 1928; from a plant cut each year, 1925–1927.

A. Terminal growing-point. B and C. Unfolding fronds. D. Petiole base 1927, with basal growing-point.

a large frond (the one cut), and a thin base, from a smaller frond cut later in the same year.

Another way in which additional fronds may arise is seen in fig. 1. Since the cutting began, one of the older growing-points has developed into a branch which has produced fronds.

The effects of cutting Bracken at the right season lead to the following results :—

(1) The fronds are removed at the stage when their formation has used up the greatest amount of food reserve in the rhizomes, but before they themselves have time to replenish the loss. At Boghall Glen this date is about 1st July, when the fronds have been eight to ten weeks above ground.

(2) Continued cutting gradually exhausts the rhizomes till the older parts die away, and the growing-points produced become smaller each year, so that any fronds arising from them are smaller.

(3) The continuous rhizome system is broken up by the decay of older parts, hence the fronds arise from detached groups of branches nearer the surface, and the supplies of water, etc., from the deeper soil layers are cut off.

(4) Cutting induces development of buds that would normally remain dormant for a year or longer.

A Seed-borne Disease of Clover (Trifolium repens L.).

By N. L. Alcock, F.L.S. and M. S. Martin, B.Sc.

(With Pl. I.)

(Read 19th January 1928.)

The value of clover in pasture has been recognised for a long time—for at least two hundred years (5, 33). But the special value of our Wild White Clover that grows on the best old pastures and upland grazings in England and Scotland has only been appreciated during the last twenty or thirty years, and will always be associated with the name of Professor Gilchrist and Cockle Park (15). Much grassland was turned to arable during the war and is now returning to pasture, and the value of Wild White Clover as a component of the new herbage is admittedly great (19, 22). The importance of the source, the healthiness, and the strain of the seed used is gradually being recognised. Seed-borne disease is a factor that must be considered (18, 29, 30).

In samples of seed of *Trifolium repens*, L., imported into Britain from Central Europe and from New Zealand in 1927, there frequently occurred clover seeds apparently affected with a specific disease. These seeds were characterised by their peculiar colour—a grey-pink—which, on examination under a low magnification ($\times$ 16–20), proved to be due to the presence of the mycelium of a fungus occurring in shining flecks on the surface of the seed coat. Associated with this characteristic symptom were brown, slightly depressed areas on the affected seeds. The infestation was specially characteristic of parcels of White Clover imported from New Zealand in 1927, in which diseased seeds occurred to an extent as great as 4 per cent. by weight. The diseased seeds were quite readily extracted from samples. Affected seeds extracted from a sample purporting to be Kentish Wild White Clover, but which was proved in legal proceedings to be seed of New Zealand origin, were submitted by the Seed Testing Station of the Board of Agriculture for Scotland to the Board's Pathological Department at the Royal Botanic Garden in

November 1927. The seeds were found, on examination, to be heavily infected with a fungus, the mycelium of which formed a loose mat under the seed coat of each seed (fig. 1).

The seeds were washed in mercuric chloride (1 : 1000) in order to destroy any adventitious spores on the outside, and then rinsed in sterile water. Whether the treatment affected the germination is uncertain. They germinated very badly, but probably that was owing to the disease. In any case the mercuric chloride was of no avail against the disease.

On 9th November some of them were planted on agar slopes and some on agar in Petri dishes for the purpose of obtaining cultures of the fungus. The medium used was oat agar.

The mycelium grew out readily, and began quickly to give concentric rings of small sclerotia which were at first white and studded with drops of water. By 24th November, *i.e.* in sixteen days, these sclerotia were fully formed and had become black and slightly warty. A few of the seeds were also damped after being washed and left in a sterile tube, where the sclerotia formed freely on the seeds themselves.

By 4th January the perfect stage began to appear in several of the cultures. The apothecia were produced in fair quantity, and the sclerotia did not seem to require a resting-stage. Some of the sclerotia not yet germinated were taken out of the tubes and were placed on sterilised wet sand and others on sterile wet cotton-wool. After the sclerotia were placed on the sand the apothecia appeared in from ten days to three weeks. The entire time from the culture of the mycelium in the seed to the apothecia was from 9th November to 4th January, on which date the first apothecia were seen. The apothecia were small, the discs ranging from ½ to 2 mm. Each sclerotium threw up several stipes ranging in number from 1 to 14 (fig. 3).

At first short brown processes appeared on the sclerotia. These were rather dark brown. As they grew, they became paler and swelled at the tip—the latter at this time being the darkest part. Presently the club-shaped end opened into a funnel-shaped dark cup. After a time this cup opened out and became much paler in colour, especially the upper surface. The disc at full maturity was saucer-like or nearly flat, the colour of the spore-bearing surface becoming very

light pinkish buff, varying from a yellow to a pinkish shade. When fully grown, the stipes averaged 4–5 mm. in length, $\frac{1}{2}$ mm. in breadth, with the discs of the apothecia ranging from $\frac{1}{2}$ to 2 mm. across. The almost constant length of the stipes is attributed to the fact that they arose from sclerotia placed on top of the substratum. Had the sclerotia been buried at varying depths there would have been a corresponding variety in the length of the stipes (4). The average size of the asci in the hymenium is 186 μ, and that of the spores 16·8 μ (fig. 4).

These apothecia that were grown on wet sand threw a great many spores against the lid of the Petri dish, the lid becoming almost milky-looking with the deposit. From these spores many more cultures were made successfully, and again produced the apothecia.

Infected seeds placed on filter-paper by Dr. Pethybridge at Harpenden (20th May 1928) produced sclerotia on the seeds, and by 28th June small apothecia appeared on the sclerotia. Similar sclerotia on the seeds themselves produced apothecia in Edinburgh on wet sand this summer. These apothecia that appeared on the sclerotia on the seed coat were in both cases very small (fig. 2, reproduced by kind permission of Dr. Pethybridge); those appearing from seed being even smaller than those that grew from sclerotia obtained in culture. In culture the mycelium produced was white, glistening, and of a sclerotinia type. After some weeks the agar tubes, full of small sclerotia, began to have small white dots on the mycelium. On examination these were found to be formed of the typical micro-conidia that have been described by other writers as occurring with the fungus *Sclerotinia trifoliorum* in culture and also in the field (4, 18, 31). Concurrently, a number of samples of English Wild White Clover were examined—at least twenty samples from many parts of England—but in no case was the sclerotinia found. Many cultures also were made from these samples, but the disease did not appear.

A series of experiments were carried out on the infection of clover plants, and these are still in progress. Pots of English Wild White Clover and New Zealand White Clover obtained from the Seed Testing Station, Corstorphine, were grown and sclerotia planted (26th April 1928) in the soil;

both sclerotia from the disease here described, and sclerotia from clover sickness from fields round Cambridge. A pot of seedlings of English Wild White Clover grown at the Royal Botanic Garden was also tried. Apothecia from this disease growing on sclerotia were also set under the plants. So far very little result has been seen. In one instance a positive result was obtained. A healthy seed of English Wild White was grown in a tube and the apothecial spores dusted on to the first two leaves on 26th April 1928. The plant became very sickly and yellow, and presently small black sclerotia came out on the base of the stem and on the roots. From this plant a culture was obtained, and the disease recovered as far as the sclerotial stage.

The sclerotinia species attacking the seed could be regarded as a small form of *Sclerotinia trifoliorum*, or possibly a dwarf variety of it. In culture the fungus agrees with the description of *Sclerotinia trifoliorum* given by Eriksson (8) or by Coleman (4) in respect of general appearance, growth, asci, and ascospores; the only divergence from published descriptions being in the size of the apothecia, particularly the disc—viz. $\frac{1}{2}$ to 3 mm. as compared with 1 to 10 mm. (Rabenhorst. Krypt. Flora. Rehm., iii, p. 817). In nature, however, the size of the disc varies very much, but the disc in this case has run persistently smaller than in *Sclerotinia trifoliorum.*

It is hoped that some more information as to the earlier stages of this disease, the method of infection, and the systematic position of the fungus will be obtained with further work.

Summary.

In 1927–28 clover seed from Central Europe and from New Zealand was received at the Seed Testing Station, Edinburgh, infected by a fungus carried by a mat of resting mycelium below the seed coat.

This fungus was placed in culture and carried out its life-history, producing first, small black sclerotia, and from the sclerotia a crop of pinkish-brown apothecial cups. The ascospores produced in the cups reproduced the fungus. The average size of the asci was 186 μ and of the ascospores 16·8 μ. The fungus agreed in size of asci, ascospores, and general life-history with that known as *Sclerotinia trifoliorum*,

but the size of the apothecia was smaller. In nature the size of the apothecia is exceedingly variable, but the cups throughout have followed a range of size below that of *Sclerotinia trifoliorum*.

One instance of reinfection and recovery has been noted.

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FIG. 1.—Seed in section showing sclerotium formed on surface and internal mycelium. (×60)

FIG. 2.—Seed with young apothecia formed on sclerotia growing on the seed. (×4)

FIG. 3.—Apothecia growing from sclerotium obtained in culture (immature). (×5)

FIG. 4.—Apothecium in section showing asci and ascospores. (×20)

Thanks are due to Dr. Pethybridge for fig. 2, and to Mr. W. Blake for figs. 1, 3, 4, and to Miss Stott for section 4.

N. L. ALCOCK.

Injection Experiments on Trees. By R. J. D. Graham and L. B. Stewart.

(Read 19th April 1928.)

These experiments, started in 1921, are still being continued as opportunity offers at the Royal Botanic Garden, Edinburgh.

The results so far obtained differ widely from those observed elsewhere (1), and this is the justification for recording results while the experiments are still proceeding.

Gramme molecular solutions of copper sulphate were used. The solution was introduced into the tree through a horizontally placed auger hole about 3 feet from the ground-level. The diameter of the auger was 1 inch, the depth of the hole varied from 2 to 6 inches according to the size of the subject. A wooden plug traversed by a metal pipe was driven tightly into the hole. The solution passed from a container by a 4-foot length of rubber tubing connected to the metal pipe. The apparatus was first tested with water which was replaced by the solution. The supply of solution in the container was renewed when necessary, fluid always being maintained in the apparatus throughout the experiment. The injections were carried out between May and July.

The path of the injected solution was traced by the penetration of the wood as seen in section. The solution passed upwards and also downwards into the roots (2). Treatment with a 3 per cent. solution of potassium ferrocyanide gave excellent results in defining the areas penetrated.

During the course of the experiments it was noted that the first traces of injury to the leaves occurred within forty-eight hours at latest from the commencement of the injection. The first leaves to be injured were on the first branch diametrically opposite the point of injection. Thereafter the crown of the tree was affected. A peculiar point was that the branch immediately above the first one affected escaped injury. The area of the crown to be first affected varied considerably, being sometimes the north side and sometimes the south.

The quantity of solution injected varied with the size of the tree, eighteen or more gallons being absorbed during the course of the experiments, which generally lasted a week. Little

difference was noted between the intake by day and by night. After the shrivelling of the leaves, fluid—solution or water—continued to be absorbed rapidly, the intake being greater at night, and much greater on the approach of showery weather.

Subject.	Age.	Annual Rings penetrated.
Salix alba	30	15–30 uniformly.
Fagus sylvestris (1)	79	10–30 uniformly. 69–79 patches.
,, ,, (2)	61	23–33 uniformly for $\frac{2}{3}$ of circumference. 36–45 uniformly for $\frac{1}{4}$ of circumference on opposite side from last. 53–61 uniformly for $\frac{2}{3}$ of circumference, overlapping the last two.
Ulmus campestre (1)	86	40–86 uniformly, heavier in outer wood.
,, ,, (2)	79	43–79 uniformly.
Acer pseudo-platanus	69	25–59 uniformly for $\frac{3}{4}$ of circumference. 63–69 two small patches.
Tilia europaea	30	1–30 localised around auger hole. 1–11 opposed to above area.
Pyrus Malus	21	6–21 uniformly.

The injured specimens were not killed outright. In the injected Acer and in an Aesculus rapid development of dormant buds gave rise to new leaves.

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On the Occurrence of Schizocotyly in certain Ranunculaceous Seedlings. By Elizabeth W. Miller, B.Sc.

(Read 17th May 1928.)

Material.—The seeds of various Ranunculaceous genera were sown in soil in ordinary four-inch pots about the middle of November. The cotyledons appeared above ground in from thirteen to twenty-one days according to the genus, and at the end of the month the first seedlings were removed and fixed in a fluid of the following composition :—

Absolute alcohol	6 c.c.
Formalin	3 c.c.
Glacial acetic acid	1 c.c.

The occurrence of seedlings with three cotyledons was noticed in the sowings of *Delphinium cashmirianum*, *Thalictrum minus*, and *Nigella damascena*, while all the other species had normal seedlings. Only one tricotyledonous specimen was found in each of the *Thalictrum* and *Nigella* sowings, but about 25 per cent. of the *Delphinium* seedlings were abnormal, and of these some were fixed at intervals as they attained sufficient size, while the rest were left in order to watch the subsequent development. Unfortunately, probably owing to the cold and to overcrowding, the latter withered before the first foliage leaves appeared.

Method.—The seedlings were embedded in paraffin, and the sections (10 μ thick) were stained with the gentian-violet–light-green combination, which showed good differentiation in spite of the small amount of lignified tissue present in such young stages.

Delphinium cashmirianum.

Lubbock (9) has described the external features of several species of *Delphinium*, including *D. Staphysagria*, *D. elatum*, *D. hybridum*, *D. cardinale*, *D. nudicaule*, *D. consolida*, and *D. Ajacis*. Work has been done on the germination of *Delphinium* by Dickson (4). Lenfant (8) studied four species anatomically, namely, *D. Ajacis*, *D. consolida*, *D. Staphysagria*, and *D. elatum*,

and to these Sterckx (11) added *D. nudicaule,* while *D. luzulinum* and *D. formosum* were investigated by Miss Thomas (12). Both Lubbock and Lenfant report the occurrence of schizocotyly in varying degree in *D. Ajacis.*

Delphinium cashmirianum possesses features similar to other species of the genus, but for purposes of comparison the struc-

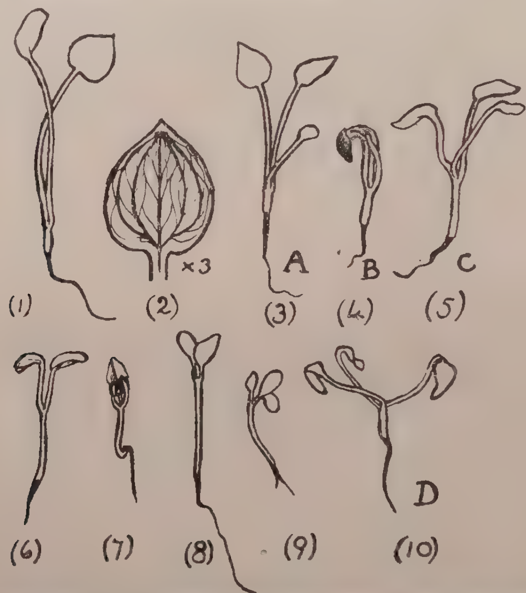

FIG. 1.—Seedlings of *Delphinium cashmirianum, Thalictrum minus,* and *Nigella damascena.* (1) Normal *Delphinium.* (2) Cotyledon of normal *Delphinium* to show veining. (3), (4), (5), and (10) Tricotyledonous *Delphiniums.* (6) Normal *Thalictrum.* (7) Tricotyledonous *Thalictrum.* (8) Normal *Nigella.* (9) Tricotyledonous *Nigella.* (All nat. size, except (2).)

ture of the normal seedling will be briefly described here. For convenience it is described from above downwards.

Fig. 1 (1) and (2) show the form of the seedling and the veining of the cotyledon ; this veining was constant (as far as could be seen) for the tricotyledonous specimens also. Three vascular bundles enter the petiole from the lamina, and coalesce at the base of the lamina to form one strand. Very soon this strand takes on the typical Ranunculaceous " double-bundle " arrangement, each double bundle consisting of one protoxylem, and, diverging from this, two groups of metaxylem each capped with a group of phloem elements. This structure is main-

tained until the cotyledon tube is reached—in this species the petioles of the cotyledons are concrescent for a considerable distance above the cotyledonary node, but the tube is short compared to that in *D. nudicaule*, and does not obstruct the exit of the plumule as in that species.

The transition belongs to Van Tieghem's third type (13). As is so common in cotyledons and early foliage leaves, there is marked asymmetry in the entry of the cotyledon traces, one coming in later and more obliquely than the other. The transition is high, as is the rule for slender seedlings, and the diarch structure characteristic of the root is attained quite near the top of the hypocotyl, although the pericycle and endodermis do not become clearly defined until the external collet is reached. In seedlings with a high transition it is clear that the internal collet is quite independent of the external collet, as Sterckx explains so carefully. Plumular xylem elements make their first appearance just below the top of the hypocotyl, midway between the protoxylem poles. Few at first, they increase in number to a maximum when a complete xylem plate crosses the centre of the stele, then they decrease and finally disappear, leaving the poles quite separate again.

The first abnormal seedling (A) to be examined was one which had grown fairly large, and two of its cotyledons were the same size, while the third was little more than half their length, its lamina being proportionately small (fig. 1 (3)). Sections showed its anatomy to be as follows. One of the large cotyledons (*b*) was normal, and had a double bundle near the top of its petiole ; the other (*a*) had a single bundle slightly larger than half a " double " one ; the small cotyledon (*c*) had a single bundle of about the same size as that of (*a*). The petioles of (*a*) and (*b*) joined by one edge considerably above the hypocotyl—this corresponds to the beginning of the cotyledon tube in the normal seedling. The petiole of (*c*) approached slowly at first, then came in at an angle of about 60° (so that the bundle was cut obliquely), and gradually joined up with the free edges of (*a*) and (*b*). When the angle of entry became more acute, it was seen that the bundle of (*b*) was the first to become double, (*c*) divided next, and (*a*) was the last to divide (fig. 2 (1)). At the stem-apex the petioles of (*a*) and (*c*) together equalled in breadth the petiole of (*b*), and the junction of (*a*) and (*c*) gradually disappeared

so that one cotyledon petiole was formed having two bundles, each a double one, and an unequally bilobed exterior.

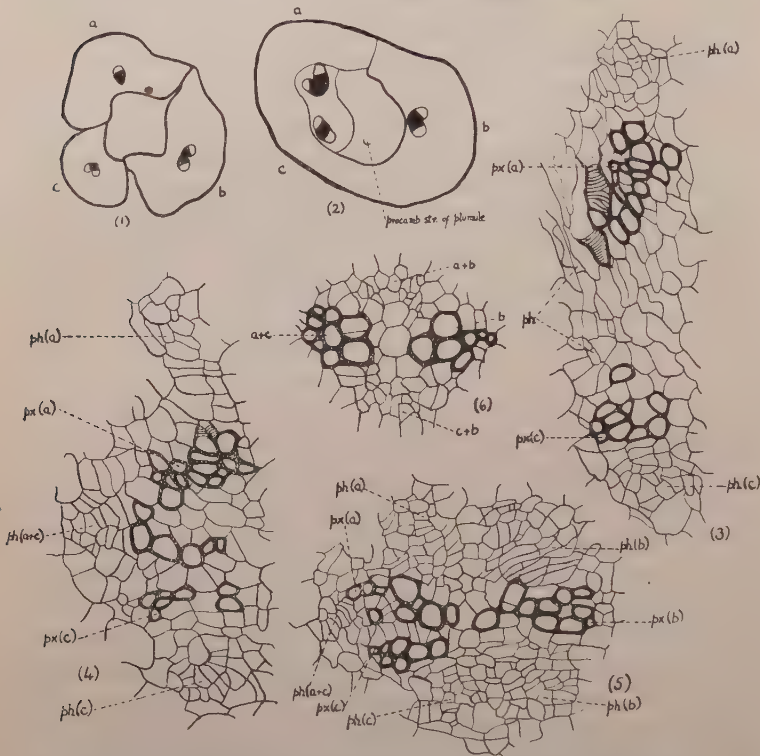

FIG. 2.—Tricotyledonous *Delphinium* (A). Letters denote bundles. (1) Semi-diagram of cotyledon tube; (*a*) has not divided, (*b*) and (*c*) are double, the division of (*c*) being later than that of (*b*). ×35. (2) Semi-diagram of cotyledonary node; bundle of (*c*) approaches that of (*a*), and the half-bundles are rotating; plumular procambium is present. ×35. (3) and (4) show convergence of bundles (*a*) and (*c*); the neighbouring half-phloems of (*a*) and (*c*) are not clear in (3), because (*a*) goes in towards axis at an angle; they are clear in (4), having joined and been pushed outwards by the convergence of xylems (*a*) and (*c*); rotations not complete. (3) ×270. (4) ×300. (5) Asymmetrically triarch; rotation of xylem not complete; (*c*) approaching (*a*); phloem (*a*+*c*) becoming squashed out; main phloem groups not yet joined. ×270. (6) Diarch, but xylem (*a*+*c*) is bulkier than xylem (*b*). ×300. (Xylem, black; phloem, outlined. px=protoxylem; mx=metaxylem; ph=phloem.)

Fig. 2 shows the structure in the hypocotyl. Applying the letters for the cotyledons to their respective bundles, (*c*) approached (*a*) fairly quickly, and at the same time all the

bundles rotated normally. The phloems from the neighbouring halves of (*a*) and (*c*) joined and were pushed outwards, the remaining phloems behaving normally and joining with the corresponding halves of (*b*). For a short distance (450 μ) an asymmetric triarch stele existed, and plumular xylem and procambium were present at this stage. As the xylem of (*c*) approached that of (*a*), the phloem between them (always smaller in quantity than the other two phloem groups) became obliterated, and finally about one-third down the hypocotyl the stele became diarch. The remainder of the hypocotyl was normal in structure, save that the xylem patch, which consisted of (*a*) and (*c*), was bulkier than the other, and this was found throughout the root as well.

The second seedling (B) was very young, and its cotyledons were still recurved and enclosed in the testa of the seed (fig. 1 (4)). Two of the cotyledon petioles were joined fairly far up, but the third was free until near the cotyledonary node; in section all three were much about the same size until the cotyledon tube was formed, when (*a*) and (*b*) decreased in size until together they were equal to (*c*), giving the appearance of two petioles, one of which had two double bundles (fig. 3 (1) and (2)); (*b*) was the last bundle to become double. Fig. 3 (3) and (4) show how (*b*) approaches (*a*). Their union resembles what took place in seedling (A), an asymmetric triarch structure being achieved, which lasted throughout the hypocotyl. Plumular procambium was present in quantity, as well as a few xylem vessels belonging to the plumular system, and these retarded the junction of the cotyledon phloems (*b*) and (*c*). At the base of the hypocotyl (*a*) and (*b*) began to rotate towards one another, and the intermediate phloem was obliterated, giving a diarch structure in the true root. At first the pole ($a+b$) was bulkier than (*c*), but further down the root this difference disappeared.

Had this seedling been a little older, it is possible that the cotyledons (*a*) and (*b*) would have separated, and that there would have been very little difference in the size of the cotyledons; although (*b*) might possibly have been a little smaller.

The third seedling (C) had a long hypocotyl, and three cotyledons of equal size (fig. 1 (5)). This seedling was halved for purposes of embedding, and unfortunately the upper half

showing the transition was lost. The part which remained, however, belonging to the base of the hypocotyl and the root,

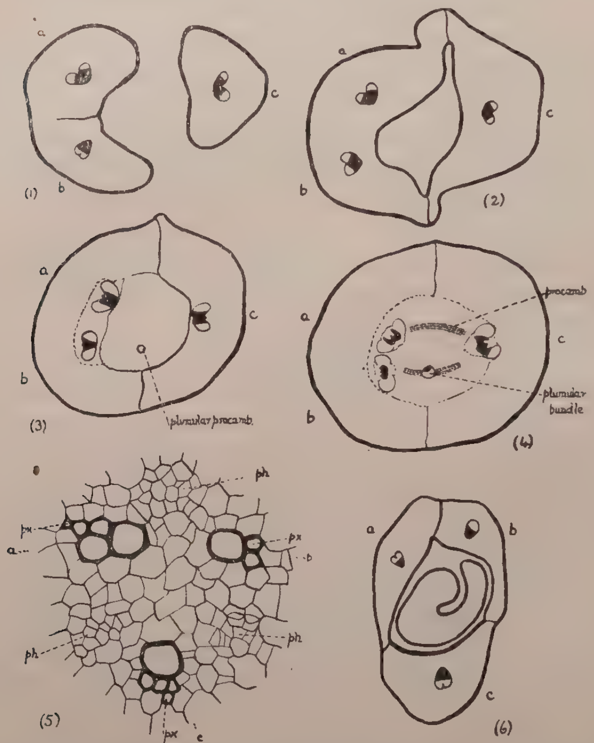

FIG. 3.—(1) to (4) Tricotyledonous *Delphinium* (B). (1) Semi-diagram of cotyledon petioles; petioles (*a*) and (*b*) have joined; note late division of bundles in (*b*). ×35. (2) Semi-diagram of cotyledon tube; all bundles are double; join between petioles (*a*) and (*b*) not visible. ×40. (3) Semi-diagram of cotyledonary node; protoxylem becoming exarch, (*b*) approaching (*a*); plumular procambium present. ×35. (4) Semi-diagram of just below cotyledonary node; bundles come in at an angle, and therefore appear longitudinal; phloem ill-defined; much procambium present as well as a plumular bundle; (*a*) and (*b*) almost joined; rotation advancing. ×40. (5) Tricotyledonous *Delphinium* (C). Structure at collet—similar throughout root; symmetrically triarch. ×270. (6) Tricotyledonous *Delphinium* (D). Semi-diagram of cotyledon tube at stem-apex; originally all three petioles were equal, but (*a*) and (*b*) are now smaller; bundle (*c*) is the first to become double, then (*a*); (*b*) is still single; petioles (*a*) and (*b*) join before joining (*c*). ×35.

showed a very symmetrical triarch stele which gave no signs of reduction to diarchy in the root. Fig. 3 (5) shows one of the xylem groups (*a*) slightly larger than the others, and (*a*)

and (*b*) have a slight tendency to point towards one another, but this was not so marked in the root.

A fourth seedling (D) was taken at a later date (fig. 1 (10)). The three cotyledons were of the same size, and externally, therefore, (C) and (D) were alike. Sections showed that at first the petioles were equal in size, but, about half-way between the lamina and the cotyledonary node, two, (*a*) and (*b*), approached one another, and (*c*) was much larger than (*a*) or (*b*). Just above the stem-apex (*a*) and (*b*) joined by one edge, and at this stage none of the bundles were double. Fig. 3 (6) shows the structure of the cotyledonary tube at the stem-apex ; (*c*) was the first to have a double bundle, then (*a*), and (*b*) was the last to divide. This was the case in all the abnormal *Delphinium* seedlings—the first cotyledonary bundle to become double belonged to the normal cotyledon, but there was no such regularity in the order of division of the other two. In seedlings (A) and (B), the cotyledon with the smaller vascular supply was the next to have a double bundle, but in (D) the corresponding cotyledonary bundle was the last to divide. The vascular structure in the hypocotyl resembled to a certain extent that in the first two seedlings, but it was complicated by the presence of much plumular xylem. The bundles (*a*) and (*b*) approached one another fairly quickly, at the same time approaching the centre of the stele, but (*c*) lagged considerably in its approach to the centre. The structure in the hypocotyl was asymmetrically triarch at first, becoming more symmetrical towards the base, and xylems (*a*) and (*b*) joined and parted again more than once. Pole (*c*) was the largest, but as it decreased in size it joined with the other two, to give a continuous xylem plate for a while. Then (*c*) separated again, and the phloem between (*a*) and (*b*) became smaller, remaining thus till the collet. At the top of the root (*a*) and (*b*) began to converge, and a diarch structure slowly appeared. This transition to diarchy was late in appearing, not taking place until some distance below the collet.

Since the above investigations were made, some seedlings grown from Sutton's Mixed Annual Larkspur seeds have been found showing all stages of schizocotyly to true tetracotyly. These are of interest, since in previous cases of schizocotyly a complete series of stages to tetracotyly has usually been found. It has not yet been possible to examine

the anatomy of these seedlings, but it is hoped that this will be done soon.

Thalictrum minus.

The external features of this species were described by Lubbock (9), while Sterckx (11) and Mansion (10) give anatomical details for this and other species. Four other species of *Thalictrum* are dealt with by Miss Thomas (12).

The normal seedling is remarkable for the late division of the cotyledon bundle as compared with *Delphinium*. There is no cotyledon tube, and there is no sign of double bundles until the cotyledonary node is reached. The seedling examined was not very old (fig. 1 (6)), but the stem bundles had already made their appearance, due to the fact that in *Thalictrum* the first foliage leaves appear very early. The transition is high, being completed very soon after the cotyledonary node, and as usual one bundle completes its rotation before the other. The hypocotyl is long, and for three-quarters of its length has the vascular structure typical of a diarch root (fig. 4 (1) and (2)).

The tricotyledonous seedling was very young, the cotyledons being still enclosed in the seed testa, but their petioles were free (fig. 1 (7)). Fig. 4 (3) to (6) show the structure of the cotyledons down to the cotyledonary node. In the testa, (*b*) had the largest number of procambial strands, therefore it is probable that this was the oldest cotyledon ; likewise (*b*) was the first to show the convergence of all the bundles to form one main central strand. Subsequent sections, however, showed that (*c*) was the extra cotyledon, and at the stem-apex (*c*) had the smallest petiole. Division of the bundles was later than in the normal seedling, there being no sign of double bundles at the cotyledonary node ; (*a*) was the first to become double, thus corresponding to the abnormal *Delphiniums*. All the bundles came in very obliquely, and the union of (*b*) and (*c*) was not clear. The phloem between them was slight and quickly obliterated, and the triarch appearance was asymmetrical and of short duration. It was noticeable that (*a*), the normal cotyledon, had completed its transition early, in marked contrast to the abnormal *Delphiniums*, where the corresponding bundle was the last to complete its rotation. Phloems (*b*) and (*a*) had joined for some considerable time

before phloems (*c*) and (*a*), the union of the latter taking place about half-way down the hypocotyl, when the normal diarch structure was completed. No further changes occurred in the

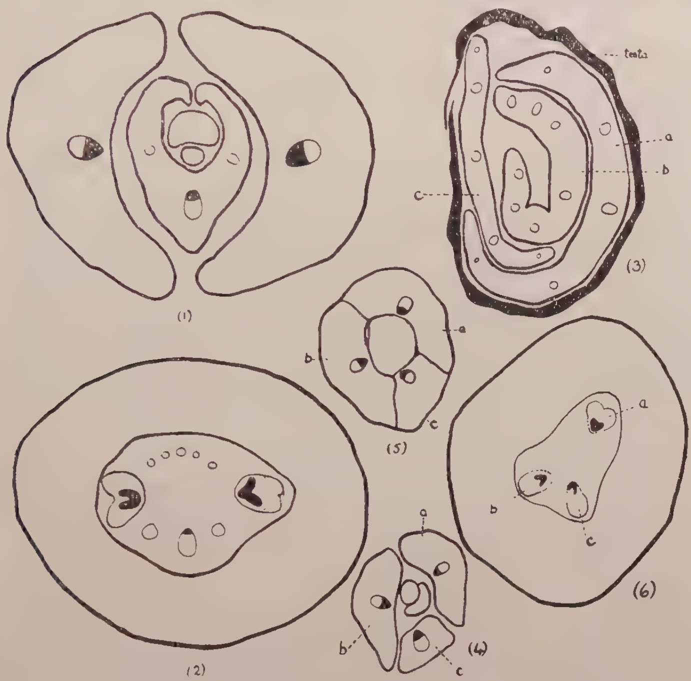

FIG. 4.—(1) and (2) Normal *Thalictrum minus.* Diagrams from camera lucida drawings. (1) Stem-apex and cotyledon petioles; all bundles single. ×35. (2) Just below cotyledonary node; bundles beginning to split—oblique due to angle of entry; plumular bundles present. ×35. (3) to (6) Tricotyledonous *Thalictrum.* (3) Cotyledons in testa; (*b*) has the largest number of procambial strands. ×35. (4) Stem-apex—single bundles. ×35. (5) Cotyledonary node. ×40. (6) Bundles (*b*) and (*c*) converging, beginning to divide, oblique. ×60.

passage to the root, save the normal changes at the external collet.

Nigella damasceña.

The external appearance of this and other *Nigella* species has been dealt with by Lubbock (9). The seedling anatomy of this and other *Nigella* species has been studied by Miss Thomas (12), and has also been most fully described by Sterckx (11), since he used it as his type for the Ranunculaceous seedlings; but Sterckx rejects the theory of xylem

rotation from the stem to the root position (*i.e.* centrifugal to centripetal), and describes the structure in connection with his theory of contacts between the xylems of the root, the cotyledons, and the stem. As the seedlings described above have been treated from the standpoint of the rotation theory, it will be as well to deal briefly with *Nigella* also from this standpoint.

The normal seedling (fig. 1 (8)) had the lamina of its cotyledons removed previous to embedding, but sections showed that even near the top of the petioles the bundles are typically double—even more so than in *Delphinium*. There is a fairly long cotyledon tube, and, although the transition is high, it is slower in beginning than in *Delphinium*. For some distance below the cotyledonary node (250 μ) the bundles show little sign of rotation; they approach the centre of the stele slowly, and well-defined stem bundles make their appearance, as usual one being more advanced than the other, and having the effect of retarding the union of the phloems on that side (or rather perhaps it forces them apart); diarchy is achieved one-third of the way down the hypocotyl. Much plumular xylem complicates the structure, but when it vanishes the two poles are left separate.

In the schizocotylous specimen, as can be seen from fig. 1 (9), one cotyledon was small and separate, while the other had a united petiole but two laminae. As before, the laminae were removed prior to embedding, but it would have been better had this not been done; for the first sections showed only two petioles (as was to be expected), and each contained only one double bundle. Therefore the vascular systems of the two halves of the bifid cotyledon must have united very early. One (the "double") petiole was larger than the other at first, and grooved on its inner side; but it very soon became the same size as the other, and by the time the cotyledon tube was reached there was no difference between them. The bundles of the "double" petiole were perhaps slightly larger than the others; they contained more xylem but less phloem. They, too, soon became of normal size, and the rest of the seedling resembled the normal seedling except for the smaller development of plumular xylem in the hypocotyl, since it was a younger seedling. The larger amount of xylem in the "double" petiole is doubtless due to the larger size of the

cotyledon it has to supply, and the smaller amount of phloem suggests that, although the bifid cotyledon must have been manufacturing more food substances than the normal cotyledon, it must have been using up a greater proportion, and so there would be less to store. The food manufactured by the cotyledon would be the source of energy for the greater number of cell divisions, resulting in the bigger lamina.

Discussion.

The occurrence of schizocotyly in Angiospermous seedlings has long been known, and there have been numerous cases mentioned by various writers, but the anatomical study of such seedlings has been neglected until recent years. R. H. Compton (2) gives a list of previous cases mentioned, at the beginning of his paper on "Syncotyly and Schizocotyly." According to him, true polycotyly, except for its presence in the Gymnosperms, is rare, and in the Angiosperms is found only in *Persoonia* (Proteaceae), *Nuytsia*, and *Loranthus* (Loranthaceae); other examples are now known to be the result of greater or less division of the cotyledons. Miss Thomas (12), however, describes *Pittosporum crassifolium* as a true polycotyledon, having four cotyledons, although tricotyledonous seedlings have also been found. This writer reports a few other occurrences of schizocotyls in the Cruciferae.

Holden and Bexon (6) have described the structure of polycotylous seedlings of *Cheiranthus cheiri*, and from their work conclude that cotyledon increase may arise either by cotyledon fission, by dichotomy of the growing point of the cotyledon, or (more doubtfully) by downward displacement of one or more epicotyledonary leaves.

Miss Bexon (1) further investigated the anatomy of some schizocotylous seedlings of *Centranthus ruber*, and reached the same conclusions as Hill and de Fraine (see below).

Lee (7), in his paper on the Compositae, mentions several stages of schizocotyly in *Dimorphotheca pluvialis*, where the extra cotyledon in the tricotyls is undoubtedly derived by fission.

M. Lenfant (8), as already mentioned, found all stages of schizocotyly in *Delphinium Ajacis*.

Hill and de Fraine (5), in connection with their work on Gymnosperm seedlings, have classified cotyledons thus, " on the first organisation of root structure, whether it appears high or low in the hypocotyl " :

(1) Vascular structure of cotyledon gives rise to one pole of root structure—Whole cotyledon.
(2) Vascular bundles of two adjacent cotyledons give rise to one pole of root structure—Half cotyledon.
(3) Trace of seed-leaf plays no part in transition phenomena, but joins on to any adjacent vascular tissue in no definite fashion—Subsidiary cotyledon.

The boundary between a whole and a half cotyledon is, however, not well defined, and there is a constant succession of stages between the two. The species studied above are a good example of this. The *Nigella* seedling was at the most elementary stage ; the lamina of one cotyledon was deeply bifid, and this had only a slight effect on the vascular system. The *Thalictrum* seedling was at the next stage of vascular structure, consisting of one whole and two half cotyledons ; the extra strand very quickly joined with that which supplied the other half cotyledon, and the intermediate phloem was not distinct, although the xylem was triarch for a short distance.

The *Delphinium* seedling (A) showed the next stage ; the cotyledons were obviously asymmetrical, and this resulted in an asymmetrical triarch stele which reverted to diarchy a third of the way down the hypocotyl. This seedling might be considered as having three " whole cotyledons " if judged by the definition given above, but one was so much smaller than the other two that this seems incorrect.

In seedling (B) the cotyledons were of more equal size, but the petioles of two were joined for some distance. Asymmetrical triarchy lasted throughout the hypocotyl, diarch structure appearing at the collet. Here again the " half cotyledons " are almost " whole cotyledons."

Seedling (D) appeared to have three equal cotyledons, and the transition from a more symmetrical triarchy to diarchy occurred some distance below the collet.

Seedling (C) was symmetrically triarch throughout, and with (D) may be considered as a true tricotyl having three " whole cotyledons."

As Hill and de Fraine point out, reduction in the number of root poles frequently occurs in true polycotyls, diarchy being the final result, and for this reason they base their classification upon the first root structure which appears. On the other hand, Compton sees in the level of the change to diarchy an indication of the degree of division in the cotyledon. "Fission of the cotyledon leads to fission of the corresponding root pole, or its connection at a higher or lower level in the axis according to the degree of splitting."

That the number of leaves does have power to influence the vascular structure of the roots has been proved by Chauveaud (1921), when he found that the production of new leaves is accompanied by a differentiation of new conducting elements in stem and root. Dauphiné (3) reduced the number of leaves of a *Lupin* seedling by removing some, and found that there was a corresponding absence of certain more or less important vascular elements in the root. These facts show that the vascular system is undoubtedly plastic, at least in the young stages. Another writer states that "schizocotyly is associated with a vascular acceleration which is very marked at the base of the cotyledon and the apex of the hypocotyl."

It does not seem possible to say what determines the appearance of schizocotyly. Since, so far as is known, it is not heritable, it is most probably due to external influences working on the cotyledons at a very young stage—perhaps some factor in the endosperm—or to some internal physiological disturbance; and these factors seem to be more prominent in some species than in others. It is evidently not the result of vascular splitting; the *Nigella* petiole bundles, and Dauphiné's and Chauveaud's experiments, prove that the vascular supply arises in response to the growth of the leaf—at any rate to begin with, although the future size of the leaf may possibly depend on the size of the vascular strand supplying it. The vascular structure rather lags behind the degree of splitting of the cotyledons, as can be seen in *Delphinium* seedling (D)—this outwardly resembles (C) in possessing three equal cotyledons, but whereas in (C) the whole root is triarch, in (D) there is a reduction in the root to diarchy.

The vascular supply of the extra cotyledon arises independently of any other vascular supply, the xylem probably being the earlier developed (as in the *Nigella* petiole), and is the

direct consequence of the division of the original normal cotyledon. The character of the stele of the root depends on the amount of vascular tissue evoked by the new cotyledon, and also on the time of division of the original cotyledon. If the cotyledon is late in dividing, the main vascular supply for the cotyledon will be in existence, and the new vascular strand will have neither time nor room to develop properly; therefore it will very soon join on to that already present, *i.e.* triarchy will very soon give way to diarchy. If, however, the division occurs early, then all three cotyledons will start approximately together, and their vascular supplies will have equal chances, when symmetrical triarchy will result as in *Delphinium* (C). Therefore the vascular phenomenon is essentially a contact of the new strand with the old strand, and not a splitting of the older strand or root pole to supply the new cotyledon. This is, perhaps, more readily seen if the structure is followed from above downwards, when the obliteration of the intervening phloem is apparent.

These examples of schizocotyly are of interest from another point of view, namely, the relative antiquity of polycotyly, dicotyly, and monocotyly, since they form an intermediate stage between the first two. True polycotyly is heritable, while schizocotyly (so far as is known) is not; but it is possible that in Angiosperms dicotyly is the more primitive, and schizocotyly is therefore "an instructive parallel in the course of evolution of polycotyly" (Compton). Schizocotyly would thus have become fixed as polycotyly in the course of time. This does not necessarily imply that polycotyly in the Gymnosperms arose in the same way. If polycotyly is primitive and dicotyly derived, then the cases of schizocotyly are interesting reversions to an ancestral condition. Monocotyly is a step further in this sequence, and may have arisen from dicotyly by fusion (syncotyly), which is more likely than that dicotyly arose from monocotyly by fission (schizocotyly).

Summary.

1. The anatomy of certain Ranunculaceous seedlings with three cotyledons was examined, and it was found that all stages of schizocotyly, including complete tricotyly, were represented.

2. An unusually large proportion of such seedlings was found in a sowing of *Delphinium cashmirianum*.

3. In one case at least the vascular structure lagged behind the division of the cotyledons, and a symmetrical tricotyledonous seedling had a diarch root.

4. The extra vascular elements are invoked by the division of the cotyledon, the reason for such division being unknown.

5. According to the time and degree of fission of the cotyledon, there are three possibilities for the behaviour of the new vascular tissue of the extra cotyledon: (1) It may unite with the vascular strand of the other part of the cotyledon above the internal collet; (2) it may make contact with the corresponding pre-existing root pole (below the internal collet); or (3) it may itself form a new root pole, as happens when the fission is very early. It is not correct to describe the corresponding vascular root pole as dividing to supply the two half cotyledons.

6. Once a cotyledon has divided, the further growth of the extra cotyledon depends on the number of vascular elements formed in response to this division. If few are formed, the cotyledon remains undersized; if many, it is normal in size.

7. Schizocotyly stands midway between dicotyly and polycotyly, and as in the Angiosperms the primitive structure is probably dicotyly, schizocotyly represents the course of evolution from dicotyly to polycotyly.

The work in connection with this research was carried out in the Botany Department of University College, Dundee (St. Andrews University), and I would take this opportunity of expressing my sincere thanks to Dr. Edith Philip Smith for her unfailing interest in the work and for her helpful criticism at all times.

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Light Receptors in Mesembryanthemum.

By Chrissy I. Kean, B.Sc.

(Read 21st June 1928.)

A noteworthy feature of the leaves of many species of Mesembryanthemum is the occurrence on the surface of circular translucent spots which are due to colourless areas underneath the epidermis. In leaves where these spots are not present there are, in many cases, epidermal hairs of various types. Although not at first obvious, it will be seen that the development of the former and absence of the latter are inter-related.

The species may be divided into two types: (1) where there is a layer of very small crystals of calcium oxalate in the outer wall of the epidermis, and (2) where no crystal layer is formed. *M. inflexa* is of the former type and is also characterised by having translucent spots uniformly distributed over both surfaces. Large, colourless cells, ovoid or biconvex in shape, occur throughout the mesophyll, separated from the epidermis usually by the outer palisade layer and from each other by the width of six chlorophyllous cells. These cells penetrate to the aqueous tissue which occupies the centre of the leaf and which is entirely surrounded by the mesophyll. The cell sap in these large cells is highly mucilaginous and contains a weak solution of tannin. They may therefore be called tannin sacs, and it is the absence of chloroplasts in these cells which gives the translucent appearance to the circular spots visible externally (fig. I (1)).

A slight variation occurs in *M. tigrinum.* The apical two-thirds of the leaf shows white circular areas, while the basal third shows translucent spots. The white spots mark the position of tannin sacs also, but the whiteness is due to the heaping up of the oxalate crystals in the outer epidermal wall at these areas. Frequently a single white spot covers two tannin sacs instead of one (fig. I (2)).

In these two species the epidermal cells have the same average size over the whole surface. The only noteworthy feature being that there are no stomata on the translucent areas. In *M. glomeratum* there are typical small, hexagonal,

epidermal cells intermixed with large, circular, protuberant cells. There is no crystal layer in the epidermis except in

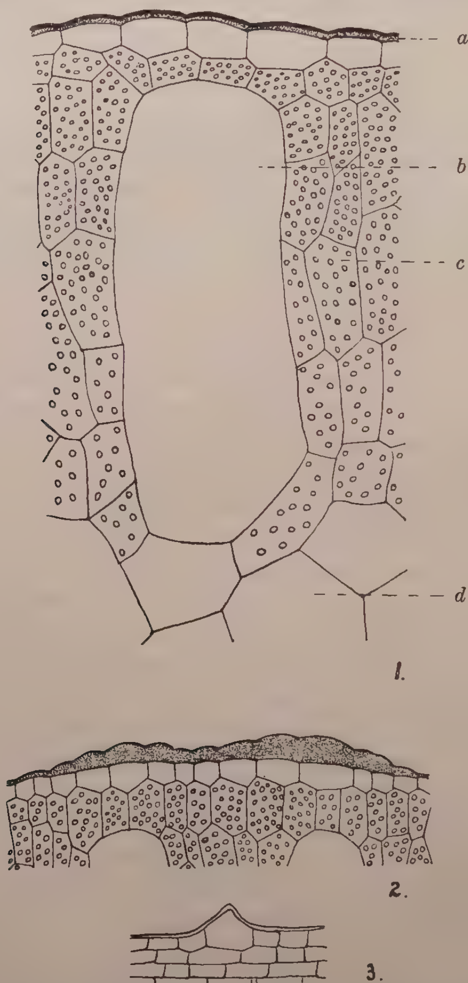


Fig. I, 1. Transverse section of a leaf of *M. inflexa*, showing a large tannin sac: *a*, crystal layer; *b*, tannin sac; *c*, chlorophyllous tissue; *d*, aqueous tissue (×160). 2. Transverse section of leaf of *M. tigrinum*, showing the thick crystal layer over two tannin sacs (×60). 3. Part of hair of *M. tigrinum*, showing an ocellar epidermal cell (×60).

these large cells which are situated above the tannin sacs (fig. II (4 and 5)). In *M. verruculatum* the epidermis has the same characters as in *M. glomeratum*, except that the large

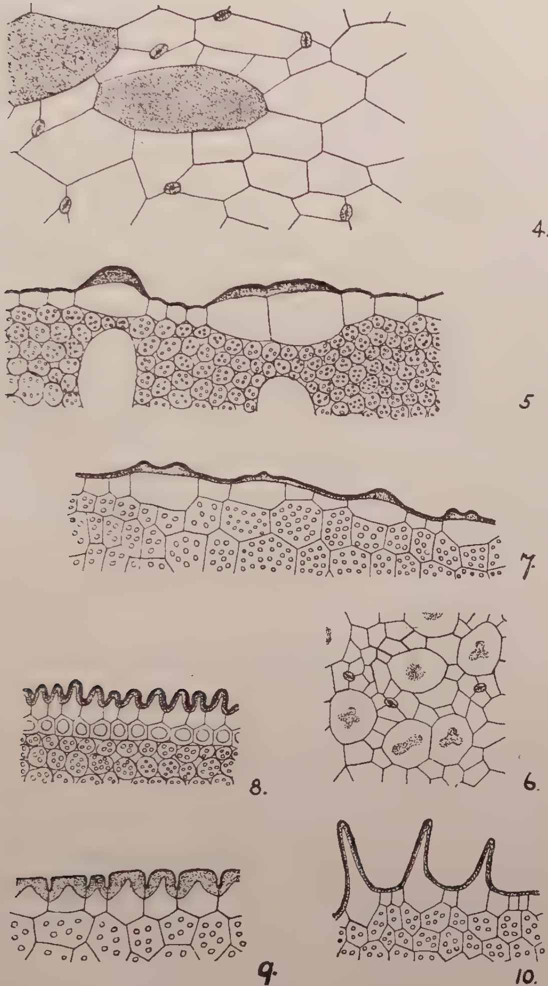


FIG. II, 4. Epidermis of *M. glomeratum*, showing normal cells with no crystals and large cells with a crystal layer. 5. Transverse section of leaf of *M. glomeratum*, with large epidermal cells above the tannin sacs. 6. Epidermis of *M. crassulinum*, showing one to four protuberances in the large epidermal cells. 7. Transverse section of leaf of *M. crassulinum*, showing epidermal protuberances. 8. Transverse section of leaf of *M. Thunbergianum*, with projecting epidermal cells. 9. Transverse section of leaf of *M. Lehmanni*. The crystal layer shows a "thin spot." 10. Transverse section of leaf of *M. Ecklonis*, showing epidermal hairs, each of which is ocellar. (All ×60.)

cells are narrow and oval. In this species, however, there are no tannin sacs in the mesophyll.

An elaboration of this type occurs in *M. crassulinum*, but here there is a crystal layer over the whole outer wall, in large and small cells alike, but in the large cells are one to five protuberances caused by a thickening of the crystal layer at certain points (fig. II (6 and 7)). Here again there are no tannin sacs.

In *M. Thunbergianum* there is a complete crystal layer, and every cell is protuberant and almost conical (fig. II (8)). There are no tannin sacs in either this or the two following species. In *M. Lehmanni*, again, every cell is protuberant but the surface is flat, and the epidermal cells are rectangular in section but are partially separated from each other. The lumen in this case is conical since the crystal layer is very thick at the angles but shows a " thin spot " at the centre (fig. II (9)). *M. Ecklonis* has typical epidermal cells intermixed with larger cells, which are drawn out into a tapering hair (fig. II (10)).

These species all have either a complete or partial crystal layer in the epidermis. *M. cultratum* is an example of the type which has no crystal layer. The outer wall of the epidermis is quite flat and tannin sacs are numerous. *M. pugioniforme*, in comparison, has no tannin sacs, and the outer wall of the epidermal cells is convex.

The relation between crystals, hairs, and tannin sacs is not clear unless the function of the tannin sacs is known. They are designated by Dannemann (1) as " water cells " whose function is (*a*) storage of water, and (*b*) captation of light. The first he proves by allowing a leaf to wilt and finding that it shows protuberances caused by the water cells, and that therefore these cells lose water less easily than the assimilatory elements. He states that they occupy mainly the undersurface of the leaf, and on account of the oblique orientation, especially in the bud, are on the best-exposed surfaces and thus transmit light to the central tissue.

That they are water-storing cells is obvious, both from Dannemann's experiment and also by observing that when the plant is insufficiently watered or the temperature is low the protuberant areas become very evident. This may be explained by the fact that the cell sap is mucilaginous.

In only a few species are the translucent spots more numerous on the under-surface, and in these cases the leaves are horizontal or nearly so, and therefore are not in the best position for captation of light. That they have a marked relation to light is shown by Haberlandt's method (2). A strip of tissue, composed of epidermis and chlorenchyma only, was mounted over a moist chamber ; a light spot was visible by reflected light, showing that the tannin sac acts as an ocellus. Several strips of tissue similar to the above were then placed with the outer surface uppermost on a piece of sensitised paper previously moistened with water, and then exposed to vertical illumination. After fixation the photograph showed dark spots marking the position of the tannin sacs.

The actual average dimensions of the tannin sacs in *M. inflexa* are 320 μ in depth and 120 μ in diameter, while the outer arc formed by the epidermis is 200 μ in diameter. The average diameter of the light spots is 150 μ. Light must therefore be condensed at the epidermis and diffracted at the aqueous tissue. The focal point is therefore within the tannin sac. The convexity of the epidermis will give condensation, but there is diffraction at this point also, caused by the crystal layer in the epidermal wall. Consequently, the focal distance is lengthened as can be shown by experiment. A piece of thoroughly soaked epidermis was mounted over a small aperture in opaque, black paper, and light applied to the outer surface. The focal point was then determined and the image on the screen measured. A piece of epidermis from *M. pugioniforme*, in which the dimensions of the cells were the same as in *M. inflexa* but there was no crystal layer, was treated in the same way. A shorter focal length was obtained, and the diameter of the image was less by one-sixth. Haberlandt's method in the case of *M. cultratum*, which has a flat surface and no crystal layer, gave the following results :—

Average diameter of tannin sacs $=240\ \mu$.
,, ,, ,, light spots $=300\ \mu$.

These results compare with *M. inflexa* as follows :—

Average diameter of tannin sacs $=120\ \mu$.
,, ,, ,, light spots $=150\ \mu$.

The two sets of results are proportionate, and the inference is that the condensation caused by the curvature of the

epidermis and the diffraction caused by the crystal layer will roughly balance; the actual diffraction obtained in the photograph is therefore entirely due to the tannin sac.

An experiment was also performed to show that the spots actually affect the acidity of the aqueous tissue. *M. obconellum* was selected in this case on account of the large size and prominence of the translucent areas. The plant has only one pair of leaves, and in one of these the spots on the upper flat surface were blackened, while the sides of both leaves were completely blackened. After several days the aqueous tissue of the leaf in which the spots were darkened gave a P_h of 5·9 and the other 6·3, showing that light passes more easily to the aqueous tissue by means of the tannin sacs. The tannin itself has no effect on the light which passes through it. It is an inactive substance and has no effect on the spectrum.

The other species with special light receptors are all modifications of the *M. inflexa* type. In *M. tigrinum* the oxalate layer is most dense in the region of greatest illumination, *i.e.* towards the apex. In this case diffraction is still greater at the epidermis. On the marginal multicellular hairs in this species is an occasional conical ocellar cell (fig. I (3)). In *M. glomeratum* the crystal layer is only found in the region of the tannin sacs, and in *M. verruculatum* in epidermal cells which alone serve as receptors. The protuberances in the large cells of *M. crystallinum* are ocellar, and in *M. Thunbergianum* each epidermal cell is an ocellus. In *M. Lehmanni* the inner layer of the outer wall is the condenser, and in *M. Ecklonis* the hair acts as an ocellus and gives a definite though faint light spot at the aqueous tissue.

References.

(1) Dannemann: Beit. zur Kenntnis der Anat. und Ent. der Mesembryanthemum. Halle-Wittenberg. 1883.

(2) Haberlandt: Phys. Anat. Eng. Trans. (1914), pp. 617–618.

Artificial Crystals in Preserved Tissue of Mesembryanthemum. By Chrissy I. Kean, B.Sc. (With Pl. II.)

(Read 21st June 1928.)

It was observed that after immersion for about two weeks in 90 per cent. alcohol, crystals appeared in the aqueous tissue of the leaves. These were of two kinds, (1) aggregate sphaerocrystals composed of elongated, rectangular plates, some with truncated ends (fig. 1), and (2) cubic crystals (fig. 2). These crystals have been observed by various workers, but have not been identified in the Mesembryanthemums. A full account of similar crystals is given for *Angiopteris evecta* by Belzung and Poirault (1). It was suggested by earlier writers that as they are abundant in tissues deprived of starch (or almost so), they replace the carbohydrate. This is not so, for they occur in tissues both when starch is present or absent. Hansen (2) shows them to be salts of calcium which are soluble in water, but give a yellow precipitate with ammonium molybdate, and are therefore crystals of calcium phosphate.

Belzung and Poirault found that after several months in 90 per cent. alcohol, whitish corpuscles 0·2 to 0·6 mm. in diameter appeared on the natural and cut surfaces of the leaf of *Angiopteris*. These corpuscles were sphaerocrystals of prisms. They found further that four days were sufficient for these crystals to appear at the natural surface. The tests made by these workers were as follows :—

(1) The crystals dissolved in cold water in ten minutes.

(2) With H_2SO_4 they gave crystals of gypsum.

(3) The addition of alcohol made a watery solution milky.

(4) On burning, the material first blackened, then swelled considerably, giving a white residue.

(5) They were insoluble in a saturated solution of neutral calcium malate, but soluble in acid calcium malate.

These tests prove the crystals to be of neutral calcium malate.

It should be remembered that in Mesembryanthemum species there are two kinds of crystals as already referred to. The sphaerocrystals measure 0·15 mm., appear after two weeks,

but do not exude at the cut surface. An attempt was made to obtain them by making longitudinal slits in the leaves and leaving them in alcohol which was allowed to evaporate slowly, but only minute crystals could be obtained in this way and not in sufficient quantity for examination. They were soluble with difficulty even in pure water, taking about a week to dissolve. This is probably due to the mucilage in which they are embedded. The cubic crystals obtained in the same way do not dissolve at all. Both give gypsum crystals with H_2SO_4, the cubic crystals after a considerably longer time. Only after the cubic crystals had been broken down by H_2SO_4 was a yellow precipitate obtained with ammonium molybdate, and that in the region of the cubic crystals only. This test, along with the fact that they are insoluble in water, shows them to be crystals of calcium phosphate. From these results it seems as if Hansen, in pronouncing the soluble crystals as calcium phosphate, was getting the molybdate reaction from phosphates in solution, or else he failed to observe the difference between the two kinds of crystals.

The sphaerocrystals were tested *in situ* by β-naphthol sulphuric acid (3). The details of this test are reproduced in "Weston's Carbon Compounds," from the Chemical News, vol. lxxv, p. 1941. On treating a section in this way, a reaction took place, giving gypsum crystals and a greenish-yellow solution which turned to bright yellow on heating. On adding a drop of cold water, a red coloration was obtained. These colour tests show the crystals to be of calcium malate. They are always embedded in mucilage and this cannot be got rid of even in the extract. An extract was made, using the method of Hilger and Cross (4), and by recrystallising, crystals were obtained similar to those in the leaf but much larger (fig. 3). The same crystals were again obtained by the method described by Onslow (5), the former method, however, being the most satisfactory, since the crystals are precipitated by alcohol; and by the latter method, incomplete precipitation only allows of the crystals persisting to the end of the separation.

With the crystals so obtained, Belzung and Poirault's tests were confirmed.

(1) The crystals were soluble though slowly.
(2) They gave gypsum with H_2SO_4.

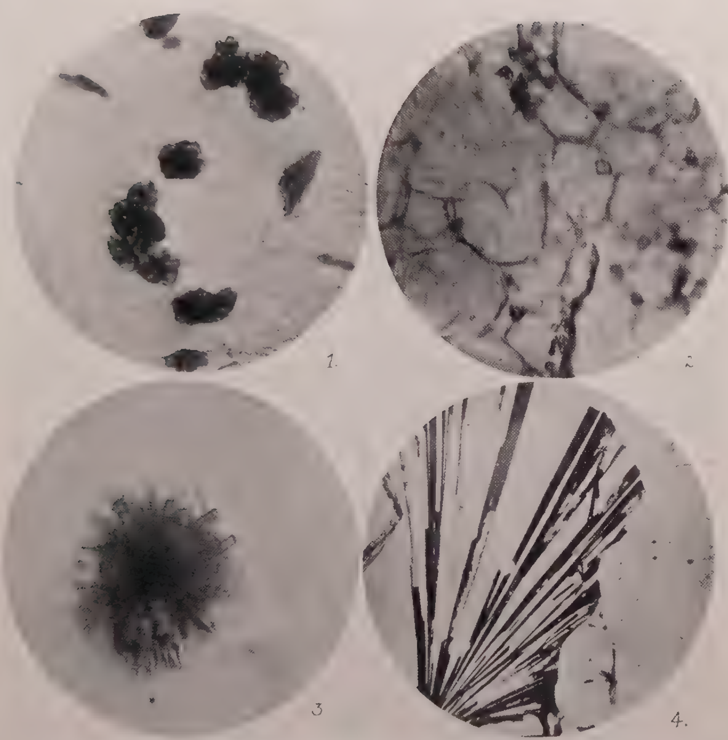


Fig. 1.—Sphaerocrystals of calcium malate in the leaf of Mesembryanthemum which has been preserved in alcohol. The preparation also shows raphides. (×120)

Fig. 2.—Cubic crystals of calcium phosphate in spirit material. (×120)

Fig. 3.—A large crystal of calcium malate obtained from an extract of the leaf tissue of Mesembryanthemum. (×160)

Fig. 4.—Crystals of calcium malate obtained by evaporating the leaf extract on a slide under a cover-glass. (×50)

Chrissy I. Kean.

(3) On adding excess alcohol, the solution became milky.

(4) Blackening and swelling occurred on burning.

(5) Sufficient material was not available to show whether the crystals were soluble or not in calcium malate.

The crystals obtained in this way, in species showing less mucilage, instead of forming a sphere, became collected together in feather-like groups, and in as pure a state as possible were deliquescent.

Summarising the above results, it is found that the artificial crystals occurring in spirit material are of two kinds, namely, cubic crystals, and aggregate sphere crystals composed of flat prisms. The cubic crystals are insoluble in water and are of calcium phosphate ; the sphere crystals are soluble in water and are of calcium malate.

References.

(1) Belzung and Poirault : Journal de Botanique (1892), p. 286.
(2) Hansen : Arbeiten des Bot. Inst. der Wurz., iii (1884–88), 102.
(3) Weston : Carbon Compounds, p. 31.
(4) Czapek : Biochemie, ii, 443–4.
(5) Onslow : Plant Biochemistry, p. 85.

Note on a Circumnutation Record.

By Marjorie L. R. Bovell, B.Sc.

(Read 21st June 1928.)

The experimental material was the shoot of *Melaleuca integrifolia*. Its growing region was 20 mm. long and its L.G.C. 0·00006, *i.e.* its growth-rate was very slow.

Darwin's glass-plate method was used, but records were taken at intervals of five and fifteen minutes respectively, instead of the longer periods of half-hour and hour and half described in the "Movements of Plants." Further, for a period of seven hours of a twenty-four hours' record the moving tip was continuously watched and its path plotted on freehand graphs.

The experiment was carried on in the Experimental Greenhouse, and automatic thermograph records were taken throughout the period of experiment. The following records were taken :—

1. A continuous record for twenty-four hours from 3.45 p.m. on 14th June through the night to the same hour on the 15th at intervals of fifteen minutes.

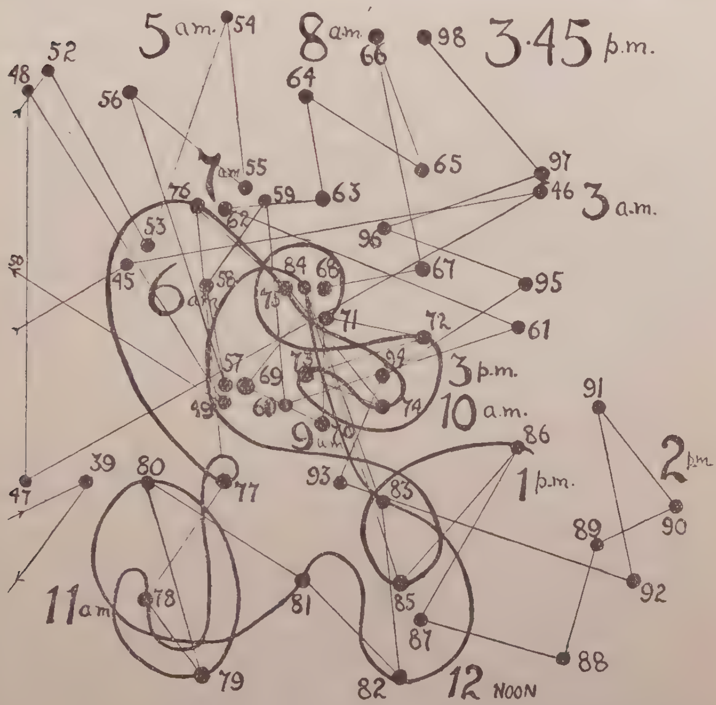
2. A record of the movement of the same shoot taken two days later from 10 a.m. to 1 p.m. at intervals of five minutes. In this case recording of the minute increments was rendered practicable by the interposition of a large bull's-eye lens between the plant tip and the horizontal glass plate ; this gave sufficient magnification to allow of the successive five-minute positions being recorded as separate points.

The following are some of the results accruing :—

The influence of light and temperature as limiting factors was demonstrated. In Record I long increments from 11 p.m. to 3 a.m. (night 14th/15th) were markedly contrasted with the short increments from 3 a.m. to 3.45 p.m. (day 15th). In the latter the limiting action of light was indicated. The same influence came out in an interesting way in Record II. For half an hour at midday there was a thunderstorm, when the light intensity fell and the temperature rose several degrees. The result of the two factors acting in the same direction is manifest in the long increments. On the afternoon of the

14th there was an alternation of fitful periods of bright sunshine and dullness, and the result was seen in the varying increments of the first part of Record I.

The next interesting feature brought out is the path followed by the moving tip from point to point. This is demonstrated particularly in the graph from 9 a.m. to 1 p.m., *i.e.* during



the period of continuous watching and freehand graphing of the moving tip.

Instead of pursuing a more or less flat curvilinear route from point to point, the tip was swung out in a series of wide semi-circles or S-shaped tracks and these approximately alternated. (See black curve in graph.)

To analyse these movements in detail was the purpose of the second record, where it was seen that the S-shaped tracks are made up of alternating reversals of clock and counter-clock movements, taking place in some cases in uniform times.

In connection with this work I have to express my indebted-

ness for facilities and supervision accorded me in St. Andrews University Botanical Department. I have also to thank my friends, former or present students of the Department, Miss M. A. Ross, B.Sc., Miss Isabella S. M'Nicoll, and Miss Frances Erica Smith, for kindly acting as successive reliefs during the period of the twenty-four hours' recording.

The Scottish Alpine Botanical Club Excursion, 1927.
By Rev. J. J. Marshall Lang Aiken, B.D.

(Read 18th October 1928.)

Choice was made of Fortingall, Perthshire, as affording ready access to Glen Lyon, a district which had lately obtained fresh botanical celebrity by the discovery of *Carex microglochin*, not hitherto reported in Britain.

The Club reached it on 2nd August through Aberfeldy, and found comfortable quarters in the modernised hotel there. Having some time at their disposal before dinner, they strolled out to take their bearings, and visited the parish churchyard close by in which a Yew (*Taxus baccata* L.) of hoary antiquity still maintained existence. "It is believed to be the oldest in Britain, and is now a mere shell, the only part remaining being the outermost portion of the old trunk, which is 56 feet in girth near the ground" (Veitch's "Manual of the Coniferae," 1900, p. 132). It is on record that, in the course of his visit to Scotland in 1770, Pennant made measurements of it, from which De Candolle made bold to estimate its term of life as not less than 2588 years! Being enclosed by a wall, its present dimensions are not easily obtained; but from a photograph taken by one of their number some idea of the vigour of one or more of its offshoots was gained.

In spite of the continuously inclement weather recently experienced throughout the island, there seemed a prospect of a change for the better, which justified the requisitioning of a car on the following day, to convey the members five miles up Glen Lyon. Next morning, accordingly, they were astir betimes, and, seated in a serviceable vehicle, entered the Glen through a deep and imposing pass, which hemmed in the waters of the Lyon in a rocky gorge beneath. The road skirted the river throughout its course; but as the ground selected for observation lay to the south, advantage had to be taken of the bridge at Invervar. On making their way down to it they were not a little surprised by a luxuriant growth of Musk (*Mimulus moschatus* Dougl.) revelling amid the herbage of a runnel by the side of the path, having dis-

carded the trig appearance assumed by it in pots and borders. Thereafter crossing the river and following a hill track above its banks, they were greeted with a display of *Gentiana campestris* L., to all seeming white, though its petals were not unfolded, which studded the meadow for a mile or more, and reached a considerable elevation as their subsequent ascent bore witness.

At the farm of Invernain a halt was called, for, though supplied with a trustworthy chart of the route to Meall Garbh, the party deemed it expedient to consult the shepherd there, who confirmed the accuracy of the information in their possession. Setting out to climb in the direction of Coire Buidheag, as indicated on the map, they left the clump of Firs on the left, and diverging westwards held up a tributary of the main hill-stream, which flowed from what was known locally as the Yellow Corrie. By so doing they gained a more gradual means of ascent; and in the course of two hours or more found themselves below its rocky ledges. The swampy ground beneath provided a typical haunt for Alpine Sedges and occupied attention for some time, as two reported associates of *Carex microglochin*, namely, *C. atrofusca* Schk. and *C. saxatilis*, L., were growing in quantity. At this point considerable ground was carefully examined with the view of gathering the Sedge in question; but in spite of their diligence, those in quest of it had to pass on unrequited.

The Corrie itself proved comparatively easy and its ledges productive, many of the plants common to the region being noted. Bird-life was scarce, and birds of prey conspicuous by their absence. Excellent pasture on the lower ground supported a heavy stock of black-faced sheep. Along the course of the stream *Lastrea montana* T. Moore grew in profusion, from the mass of which one member collected a distinct truncate form. The downward journey was uneventful though it tried the joints of all; and a pleasant run from Invervar brought the party to the hotel in time for dinner.

Among other plants sighted or gathered the following may be mentioned: *Thalictrum alpinum* L., *Cochlearia alpina* Wats., *Viola lutea* var. *amoena* Huds., *Cerastium alpinum* L., *Arenaria sedoides* Druce, *Silene acaulis* L., *Rubus saxatilis* L., *R. Chamaemorus* L., *Parnassia palustris* L., *Saxifraga stellaris* L., *S. hypnoides* L., *S. oppositifolia* L., *S. aizoides* L., *Epilo-*

bium anagallidifolium Lam., *E. alsinefolium* Vill., *Gnaphalium supinum* L., *Vaccinium uliginosum* L., *V. Vitis-Idaea* L., *Armeria maritima* Willd., *Lysimachia nemorum* L., *Polygonum viviparum* L., *Salix herbacea* L., *Listera cordata* Br., *Tofieldia palustris* Huds., *Kobresia bipartita* Dal. Tor., *Carex capillaris* L., *C. Œderi* Retz., *C. pilulifera* L., *C. pallescens* L., *C. atrofusca* Schk., *C. rigida* Good., *C. echinata* Murr., *C. pulicaris* L., *C. saxatilis* L., *Poa alpina* L., *Asplenium viride* Huds., *Athyrium alpestre* Milde, *Cystopteris fragilis* Bernh., *Polystichum Lonchitis* Roth, *Lastrea aristata* Rendle et Britten, *Phegopteris polypodioides* Fée, *Lycopodium alpinum* L., *L. clavatum* L., *L. Selago* L., *Selaginella selaginoides* Gray.

Thursday, 4th August, was regarded as an "off" day, as more than one of the party required to return home that evening. As Loch Tay was not more than five miles distant and presented attractions because of the establishment of a number of less common plants upon its shores, it was agreed to drive to Fearnan and spend a few hours there. Alighting at the pier, the party at once came upon a beautiful pink form of the Bladder Campion (*Silene Cucubalus* Wibel), Wild Basil (*Clinopodium vulgare* L.), and two stately Hawkweeds (*Hieracium gothicum* Fr. and *H. auratum* Fr.). Amid the scrub which fringed the Loch, *Thalictrum Kochii* Fr., *Circaea alpina* L., and *Galium boreale* L. were also conspicuous. They would gladly have lingered on the shore had not the sultry character of the weather forced them to seek the shade of the highway, whose ditches had been partially cleaned out but not sufficiently to frustrate the search of the botanist. In the course of a leisurely walk there were gathered a local form of Cranesbill (*Geranium sylvaticum* var. *Wanneri* Briq.) and the Marsh St. John's Wort (*Hypericum dubium* Leers), besides other plants commonly associated with the Scottish Highlands. One of the party skilled in Fern culture had the good fortune to find among the roadman's leavings a well-marked form of *Athyrium Felix-foemina* var. *polydactylum*. Among Sedges which were frequent may be named *Carex hirta* L., *C. panicea* L., *C. diversicolor* Crantz, and *C. flava* L. As there was ample time for rumination as well as for exercise, the merits of Fearnan as a place of meeting from which to make for Ben Lawers, ascend by the burn and cross the ridge to reach the Yellow Corrie, were discussed, this alternative

route having been commended by Rev. Henry H. Harvey, to whose directions the members had been greatly indebted on the previous day.

Thus ended a short and enjoyable meeting, during which the weather had proved remarkably propitious. Sunshine, however, did not lessen the sense of disappointment caused by their failure to locate the chief object of their quest.

The Reaction of the Medium in Relation to Root Formation in Coleus. By E. Philip Smith, B.A., Ph.D., F.L.S.

(Read 18th October 1928.)

The most favourable reaction of the medium for the production of roots by seedlings or cuttings is a matter of considerable practical importance. Small (2) has stated that an acid medium favours root production in cuttings, using Acetic Acid, 1 in 10,000. Hoagland (1) presents some interesting results of the effect of the reaction of the medium on rooting of Barley seedlings. He expresses his results in terms of relative weights of roots and tops at different pH values of the culture fluids, and the optimum seems to be in the region of pH 5·2. Experiments were undertaken with cuttings of *Coleus* to determine, if possible, the optimum for this plant.

The normal procedure at the Royal Botanic Garden, Edinburgh, is to root cuttings such as *Coleus* which require a moist bottom heat of 70°–75° F. in coco-nut fibre. This is an excellent material for the purpose, since it is light and porous in texture and holds a good amount of water without becoming sodden. It is possible to squeeze a few drops of moisture from the fibre as used in the frames, and on testing the pH value of this it was found to be between 4·5 and 4·7 in all cases examined. The liquid was dark brown in colour, but by using the B.D.H. Capillator the brown colour gave little trouble. It is evident, therefore, that the cuttings are normally rooted in an acid medium.

The material is very strongly buffered, large additions of acid or alkali being needed in order to change the pH appreciably. This made it unsuitable for an experimental medium, and various substitutes were tried. Pure quartz sand, washed in running tap-water for a week, was found not to affect the pH of the solutions with which it was moistened, but it proved almost impossible to remove from the roots. Glass wool was persistently alkaline, and cotton wool became sodden and did not allow access of air to the roots. It therefore became obvious that water cultures were the most suitable method in this case. Preliminary trials were made with unbuffered

tap-water, adjusted to pH 4·5, 7·2, and 9·2 by the addition of traces of hydrochloric or sulphuric acids or of sodium carbonate. The results of these experiments have been recorded (3), and it is enough to say that a pH of about 7 was found the most favourable.

The need for a closer series of buffered culture solutions led, after considerable experiment, to the adoption of the phosphate mixtures described by Hoagland (1). Certain of Hoagland's mixtures (Nos. H 26, 27, 30, and 31) were used directly, and other combinations (indicated below by letters) were made up to suit the experiments on hand. These phosphate mixtures proved very satisfactory; they were sufficiently well buffered to retain their initial pH value for several days, making it unnecessary to renew the solutions every day, and the cuttings thrived in them better than in plain water. In order to avoid the toxic effects of distilled water, the dilutions were made up with tap-water. The Edinburgh tap-water is about pH 7·3, so that the solutions were slightly more alkaline than the values given in Hoagland's paper. The pH values were determined afresh for each batch of solution, but they were very constant.

The stock solutions were:

(1) K_2HPO_4, M/2.
(2) KH_2PO_4, M/2.
(3) $NaNO_3$, M/2.
(4) NaCl, M/1.
(5) Na_2SO_4, IOH_2O, M/4.

From these the following mixtures were made up, a litre at a time, which gave two or three changes of culture, pH 2·8, 4·6, 5·2, 5·6, 6·2, 6·46, 6·8, 7·0, 7·4, 7·8, (9·2). The composition of the mixtures is given on p. 55.

The cuttings were rooted in large carefully cleaned gas jars, with about two inches of fluid. They were thus surrounded by moist air, and no flagging (with consequent retardation) occurred. The cuttings as soon as made were floated in the tank of the greenhouse where the experiment was going on, until fully turgid. It was found that this simple precaution made for greater uniformity in the results, by securing an equal start for all the cuttings. The culture solutions were lightly tinted with an indicator, which enabled departures

from the original pH to be seen at a glance. The following indicators were used: thymol blue, phenol red, brom-phenol blue, di-ethyl red, methyl orange, brom-cresol green. A concentration ten times that used in the culture pots was not toxic to the cuttings in seven days.

Phosphate Mixtures. (cc. Stock Solutions per litre Culture.)

	1.	2.	3.	4.	5.	pH.
H26	19	—	1·7	0·8	0·8	7·8
27	11	11	1·7	0·8	0·8	6·5
30	—	25	1·7	0·8	0·8	4·6
31	—	10	1·7	0·8	0·8	2·8 (+1 cc. 10 per cent. Phosphoric Acid)
A	15	10	1·7	0·8	0·8	6·8
B	10	15	1·7	0·8	0·8	6·46
C	5	20	1·7	0·8	0·8	6·2
D	—	25	1·7	0·8	0·8	5·2
AA	25	—	1·7	0·8	0·8	7·8
AB	22·5	2·5	1·7	0·8	0·8	7·4
AC	20	5	1·7	0·8	0·8	7·0
CA	2·5	22·5	1·7	0·8	0·8	5·6

The cultures all became more acid during the experiment, due to respiratory CO_2 given off from the cuttings. This was proved to be the chief source of acidity by the ease with which a current of CO_2 free air restored the initial pH. At the beginning of an experiment there was a slight amount of acid due to the leaching out of fatty acids from the cut ends of the shoots. This is correlated with differences in callus formation between the water and the fibre cultures. In fibre the cut end gives an intense reaction with Sudan III (indicating an effective wound blocking with fatty substances), and a basal meristem forms callus to a depth of three or four cells. In water the cut end shows little or no stain with Sudan III and callus formation is inhibited, but in spite of this, rooting goes on freely.

At the end of fourteen days the cuttings were removed, the roots cut off, and roots and cuttings dried to constant weight at 100° C. The results are expressed as the weight of root (in milligrams) corresponding to one gram cutting. This is not entirely satisfactory, since it is not possible to compare

the initial and final dry weights of the cuttings, but with this reservation the results are sufficiently consistent to give at least an indication of the effect of the reaction of the medium on the production of roots. The following figures give the weight of root (in milligrams), corresponding to 1 gram of cutting at the given *p*H values, after fourteen days at about 70° F. The figures are the mean of five or six experiments, each batch including 15–20 cuttings (except for *p*H 4·6, which is only one experiment).

*p*H of Medium .	2·8	4·6	5·2	5·6	6·2	6·5	6·8	7·0	7·4	7·8
Weight of Root (milligrams) .	200	1390	1127	1236	1245	1461	1286	1304	1081	1186

Another criterion which might be used is the number of root primordia produced in a given time. Counting the primordia is easy in *Coleus*, because they are visible from the outside as white points as soon as they are recognisable in section, and it was done roughly in several of these experiments. The number of roots formed varied in the same general way as the total weight of roots. While this method would indicate the effect of the reaction of the medium on the production of *root meristems*, it was felt to be less satisfactory than the other because it does not take into account the aftergrowth of the roots, which is the important thing from the cultivator's point of view.

It will be seen from these data that the optimum reaction for this plant in water culture is about *p*H 6·5. The amount of growth falls off on either side of this point, with a slight secondary peak at *p*H 7·8. This is slightly more alkaline than the value found for Barley by Hoagland.

The effect of the buffer solutions on the retention of acidic and basic dyes by the stem was tested in two ways. First, stems were allowed to stand in the various buffers for twenty-four hours and then sections made of the exposed end. Secondly, sections were cut of living material and then soaked in the buffers for the same length of time. The results from the two methods corresponded. Both Hoagland's phosphate mixtures and the B.D.H. " Universal Buffer Solution " were

used. After staining for five minutes in 0·5 per cent. aqueous Eosin (acidic) or 0·5 per cent. Toluidine Blue (basic), the sections were washed in the buffer solutions and examined under the low power. The Eosin gave a clear-cut result: at pH 7·8, 7·4, 7·0, 6·8, the stain was taken up by the walls of the new wood and the fibres, but not by the protoplasm. At pH 6·5, 6·2, 5·2, the protoplasm stained strongly and retained the stain after washing. With Toluidine Blue the stain was retained strongly down to pH 7·0, and weakly below that point. The retention of the dyes was most marked in the living cells of the pith and the interfascicular cambium. The cortex did not give so good a reaction. This points to an isoelectric point for these tissues in the region of pH 6·5–6·8, and, as both these tissues are particularly active in regeneration, suggests that a reaction near the isoelectric point of the tissue favours new growth.

The condition of the reserves had a marked influence on the rooting. The cuttings were made at random from two batches of plants, one lot growing in full light and the other in a shaded house. The first had brightly coloured leaves and short red internodes, while the reserves were mostly starch, with traces of reducing sugars. The second batch had larger and paler leaves, and longer internodes with no red colour. The chief reserves were reducing sugars. The "starch" plants produced more roots and produced them more quickly than the "sugar" plants. In all cases there was a basipetal concentration of reserves in the cutting after a few days.

Testing the two types separately showed that, while the absolute amount of root produced at a given pH was greater in the case of the "starch" plant than in the case of the "sugar" plant, the relative effect of the different reactions of the cultures was similar. Therefore in most of the experiments the material was a mixture of the two lots of plants.

Summary.

1. Stem cuttings of a form of *Coleus Blumei* were rooted in water cultures of various pH values, using dilute mixtures of phosphates.

2. A reaction of pH 6·5 was found to be the most favourable for root production.

3. An isoelectric range of pH 6·5–6·8 is suggested for the principal tissues of the stem.

4. The total carbohydrate of the stem influences the absolute amount of root produced, but the relative effects of the different media on root production are independent of this.

5. Root formation is not affected by the inhibition of callus formation in water culture.

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(2) Small, James (1923): Rooting of Cuttings in Acidic Media, Gardeners' Chronicle, ser. 3, lxxiii.

(3) Smith, E. Philip (1926): Acidity of the Medium and Root Production in *Coleus*, Nature, cxvii, 339.

The Causal Parasite of the Lily Disease.

By James Wright, B.Sc.

(Read 18th October 1928.)

In 1889 Marshall Ward (10) described in detail a disease of lilies, now usually referred to as the lily disease, and showed that the causal organism was a species of *Botrytis*. He proved beyond doubt the pathogenicity of the fungus, and showed that it conducted its parasitic existence by means of enzymes and toxins. Since the publication of that now classical paper, it has been generally assumed that the pathogen was the common saprophyte, *Botrytis cinerea*, although Ward drew attention to the fact that it exhibited a difference in size and rapidity of germination of the conidia, and also in the time elapsing from spore germination to the formation of the characteristic Botrytis "Haft-organen." As far as could be ascertained no more literature on this subject has been published.

Recently an outbreak of disease among lilies (*L. candidum*) occurred in a garden near Edinburgh. The symptoms of the malady and the characteristics of the parasite correspond in detail with the description given by Ward. A fungus received from Long Ashton, Bristol, isolated from diseased specimens of *L. candidum* grown in that locality, also agreed with the description.

Symptoms and Course of the Disease.

The external symptoms of the disease are the appearance of orange-brown specks on the leaves, stems, pedicels or buds, and thereafter the affected parts turn brown and shrivel up. The reproductive organs of the fungus appear on decaying portions and, sometime later, sclerotia, primarily white and then black, partially embedded in the tissues, are formed abundantly.

Characters of the Parasite.

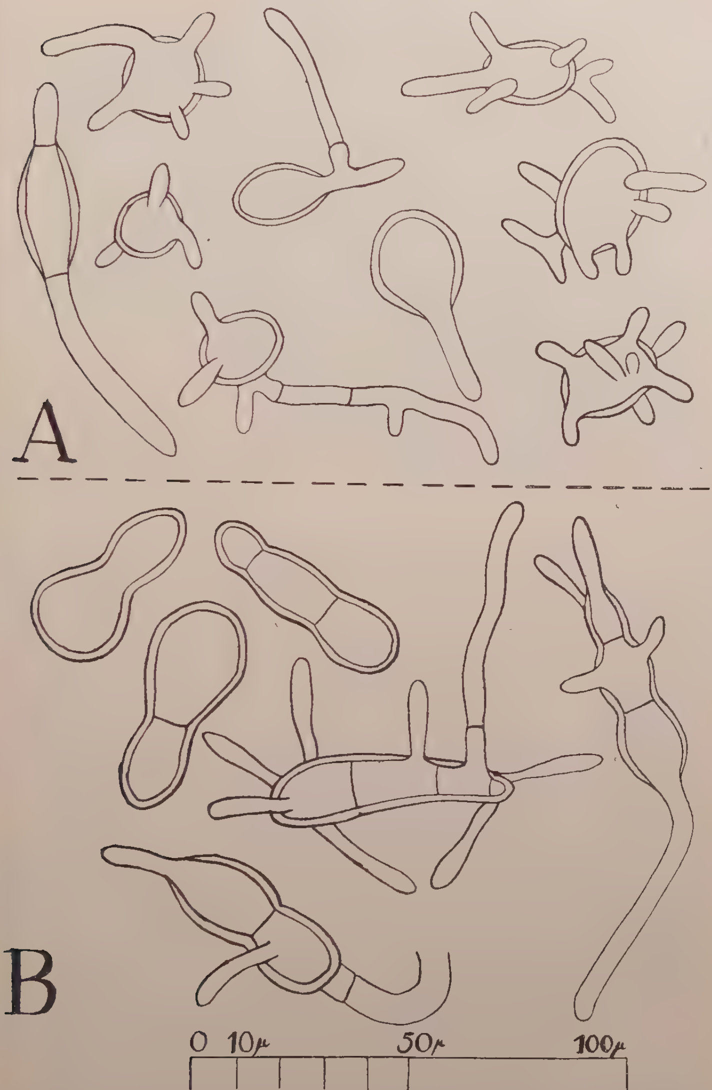
The mycelium exhibits the typical peculiarities of a *Botrytis*. It is septate, frequently branched, and varies in diameter

with the conditions under which it lives. The tips of young vigorous hyphae may be curved. Complex tassel-shaped organs of attachment are formed, but not so early, nor in such abundance as in the case of *B. cinerea*. Other important features are the fusion of hyphae, which may be observed in three-days-old cultures, and the exudation from the hyphae of droplets of a translucent nature, which were shown by Ward to contain the cellulose-dissolving ferment.

The conidiophores arise directly from the mycelium as thick, blunt, erect hyphae, full of protoplasm. They become septate and are dark brown when mature, except at the apex where they remain hyaline. They measure 12–16 μ in diameter but are more or less indeterminate in length. Under the conditions in a moist chamber, after producing a terminal head of conidia, the main axis may proliferate and produce another head, and the process may be repeated several times. On one conidiophore the successive development of ten heads of conidia has been observed. As the atmosphere dries, the conidiophore becomes flattened and twists on its axis.

The conidia are attached by short sterigmata. Primarily they are minute, globose, and colourless, but as they mature they become ovoid and the walls assume a sepia hue. The average size of mature conidia is 25–26 $\mu \times 18$ μ, but the variation in dimensions is considerable ; the range in length is 18–32 μ and in breadth 13–24 μ. On Quaker-oat media and on potato slopes, bizarre types of spores are of common occurrence. These assume a variety of shapes and may be twice as large as the normal conidia. The wall may be constricted in one or two places and corresponding cross septa appear, or one feature may occur independently of the other (fig. B).

The conidia, when placed in a drop of distilled water, tap water, or nutrient solution, usually begin to germinate at once. At first, one or two germination tubes are produced, apically or laterally, but the number is generally increased. After two days, in " hanging-drop " preparations, kept at room temperature, the number of germ tubes is found to vary from one to eight per conidium (fig. A). The number appears to be independent of the medium, but the tubes are stouter and more strongly growing in good nutrient media. A



A. Types of germination of normal conidia after two days in tap water.
B. Bizarre forms of conidia found in cultures on Quaker-oat media and on potato slopes; three spores, of which one is still *in situ*, are shown in the process of germination. (Camera lucida.)

common phenomenen is the germination of the conidia *in situ*.

The sclerotia, first white, then black, as mentioned previously, are flattened, somewhat convex structures, which vary widely in shape and size. They are very much smaller than those of *B. cinerea* and are very similar to those of *B. tulipae*. The commonest type of naturally occurring sclerotium is circular, with a diameter of one millimetre, but elliptical and more or less irregular forms are plentiful, varying in length from 1–6 mm., in breadth from 0·5–1 mm. In culture, the size of sclerotia varies with the amount of drying to which the medium is subjected. The sclerotia, on germination, produce typical conidiophores bearing typical conidia.

Microconidia were not observed, although careful observations were made over a period of three months, and it seems probable that these are altogether absent from the life-history.

Nomenclature.

Following the assumption of Ward, the first record of the disease is to be found in "The Gardeners' Chronicle" of 1881, in the form of a letter, with a reply by Berkeley (1). The fungus is reported as attacking all species of lilies, with *L. auratum* outstanding in susceptibility. Berkeley states that the fungus is presumably allied to *Peronospora*, with spores similar in shape and size to many of the large Pezizas, and names it, provisionally, *Ovularia elliptica*—"floccis hic illic nodosis, sporis magnis ellipticis laevibus." Saccardo (8) in 1886 gave the following description: "Hyphis abbreviatis hinc inde nodulosis, articulatis, e mycelio repente oriundis; conidiis ellipsoideis, hyalinis, majusculus. Hab.: In Liliorum varietatibus pluribus in Britannia." In 1888 Smith (9), on very insufficient evidence, changed the name to *Peronospora elliptica*. His scale drawings of the conidia and conidiophore correspond closely with the *Botrytis* described by Ward, who seems to be absolutely justified in arriving at the conclusion that all three fungi were the same.

In a recent paper by Dowson (3) reference is made to the lily disease, and it is stated that probably the same species of *Botrytis*, which is "certainly not *B. cinerea*," is parasitic on Spanish and other bulbous Irises. A *Botrytis* attacks lilies

in Bermuda, but in this case the pathogen appears to be *B. cinerea*. Fujikuro (5) in 1914 published an account of a Botrytis disease of *L. longiflorum* in Japan. It is noteworthy that the description corresponds somewhat with the case under record, but the fungus, which the author describes as a new species, *B. liliorum*, appears to be distinct. The conidia are pale grey and average 32 $\mu \times 27\ \mu$, with the range in length and breadth respectively 28–37 μ and 21–31 μ, and they germinate with the production of only 1–2 hyphae.

It is interesting to note that the fungus under investigation, as regards length of conidia and number of germ tubes, is intermediate in character between *B. cinerea* and *B. polyblastis*, a new species recently described by Dowson (4).

	B. cinerea.	The Lily-fungus.	*B. polyblastis.*
Average spore length .	10 μ	25–26 μ	40 μ
No. of germ tubes . .	1–3	1–8	8–13

Summing up, the lily-fungus, which possesses the diagnostic characters of the genus *Botrytis*, has the following specific features :—

1. The conidia are large, averaging 25–26 $\mu \times 18\ \mu$.
2. One to eight germ tubes are produced.
3. As compared with *B. cinerea*, it fruits poorly on synthetic media.
4. In culture, the organs of attachment are not formed so early nor in such abundance as in *B. cinerea*.
5. The sclerotia are similar to those of *B. tulipae* and quite distinct from those of *B. cinerea*.
6. There is a probable absence of microconidia.

It is deemed that the above points are indicative of a distinct species, and adopting the specific term of Berkeley and Smith, the name *Botrytis elliptica* is suggested.

Botrytis elliptica (Berk.) Wright.
Ovularia elliptica Berk.
Peronospora elliptica (Berk.) Smith.

Mycelium septate, branched, variable in diameter, with cross connections, organs of attachment, and exudation of droplets of ferment. Conidiophores arising directly from the mycelium, 12–16 μ broad, indeterminate in length, septate, proliferating, brown when mature except at the apex, where

they remain hyaline, flattened when dry and twisted on axis. Conidia in groups on conidiophores or short branches of the ordinary mycelium, attached by short sterigmata, ovoid, hyaline with sepia wall, 18–32 $\mu \times$ 13–24 μ, averaging 25–26 μ $\times 18$ μ, germinating by the production of 1–8 germ tubes; bizarre forms common on artificial media. Sclerotia first white, then black, flattened, convex, circular, 1 mm. in diameter, elliptical, irregular, 1–6 mm. $\times$ 0·5–1 mm. Microconidia absent.

Control Measures.

1. *Position.*—The disease, usually sporadic, may become epidemic if the plants receive a sudden and severe check during the growing season, as is caused by a period of cold, stormy, wet weather. As the prevailing winds at such a time are N. or N.E., the lily bed, if possible, should have a sheltered, southern exposure.

2. *Sanitation.*—All plants when they show signs of attack should be removed and burned. The disease, if allowed to run its course, will be spread by the conidia, and carried over to another year by the sclerotia which find their way into the soil.

3. *Spraying.*—Ogilvie (7) reports that in Bermuda the Botrytis blight of *L. longiflorum* var. *Harrisii* is adequately kept in check by spraying with Bordeaux mixture. Hopkins (6), however, states that in experiments at Wisconsin on the spraying of tulips for *Botrytis*, a 5–5–50 mixture caused considerable injury to the leaves and flowers, besides giving them an unsightly appearance. According to Bewley (2), the specific spray for *Botrytis* is a 2 per cent. solution of Calcium Bisulphite, which is used to destroy fungal infection on the soil surface.

Summary.

An outbreak of a Botrytis blight of lilies is recorded. The disease and the causal parasite agree with the description given by Marshall Ward. The fungus is distinct from the species causing disease of lilies in Bermuda, and also from the species parasitic on lilies in Japan, and is described as a new species *Botrytis elliptica*. A note is added on control measures which may be adopted.

The writer acknowledges his indebtedness to Mrs. N. L. Alcock for material and valuable assistance, and to Dr. Malcolm Wilson, Mycology Department, University of Edinburgh, where the investigation was carried out ; also to L. Ogilvie, Esq., M.A., B.Sc., Advisory Mycologist, Long Ashton, late Plant Pathologist, Bermuda, for his kindness in sending fungus cultures.

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(3) Dowson, W. J. : Some Fungus Diseases of Bulbs. Journ. Roy. Hort. Soc., lii (1928), 49.

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(7) Ogilvie, L. : Rept. Plant Pathologist in Rept. Dept. Agr. Bermuda for 1925 (1926), ex. Rev. Appl. Myc., vi (1927), 16.

(8) Saccardo, P. S. : Sylloge Fungorum, iv (1886), 145.

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Botanical Society of Edinburgh.

(FOUNDED 1836.)

OCTOBER 1928.

I.—GENERAL VIEWS AND OBJECTS OF THE SOCIETY.

The attention of the Society is turned to the whole range of Botanical Science, together with such parts of other branches of Natural History as are more immediately connected with it. These objects are cultivated :—

1. By holding Meetings for the interchange of botanical information,—for the reading of original papers or translations, abstracts or reviews of botanical works, regarding any branch of botanical knowledge, practical, physiological, geographical, and palæontological,—and the application of such knowledge to Agriculture and the Arts.

2. By publishing *Proceedings and Transactions*, including a List of Members.

3. By the formation in Edinburgh of an Herbarium of Foreign and British Plants, and of a Library and Museum for general consultation and reference.

4. By printing from time to time Catalogues of Plants, with the view of facilitating the study of their geographical distribution, and furthering the principle of exchange.

5. By making Botanical Excursions both in the neighbourhood of Edinburgh and to distant parts of Britain.

II.—LAWS OF THE SOCIETY.

CHAPTER I.

FUNDAMENTAL LAWS.

1. The Society shall be denominated "THE BOTANICAL SOCIETY OF EDINBURGH."

2. The object of the Society shall be the advancement of Botanical Science, by means of periodical meetings, publications, correspondence, and interchange of specimens amongst its Members.

3. The Society shall be open to Ladies and Gentlemen, and shall consist of Honorary and Ordinary Fellows, and Corresponding Members, who shall have the privilege of denominating themselves Fellows of the Society; of Associates elected under the rule Chapter IV., Section 4 hereof, and of Lady Members elected under the rule Chapter IV., Section 5 hereof, and of Ordinary Members elected under the rule Chapter IV., Section 6 hereof.

CHAPTER II.

ORDINARY MEETINGS.

1. A Meeting of the Society shall be held on the Third Thursday of every month, from October to June inclusively.

2. Intimation of all papers to be brought before the Society must be given to the Secretary and submitted to the Council ten days at least previous to the Meeting at which they are to be read.

3. Any Member may transmit to the Society Papers and Communications, which, if approved of by the Council, may be read by the author, or, in his absence, by the President or Secretary, at any of the Ordinary Meetings.

4. The following order of business shall be observed:—

PRIVATE BUSINESS.

1. Chair taken.
2. Minutes of Private Business of preceding Meeting read.
3. Report of Council read.
4. Applications for Admission read.
5. Members proposed at preceding Meeting balloted for.
6. Motions intimated at previous Meetings discussed.

7. New Motions intimated.
8. Miscellaneous Business.
9. Society adjourned.

PUBLIC BUSINESS.

1. Chair taken.
2. Laws signed by New Members.
3. Minutes of Public Business of preceding Meeting read.
4. Papers and Communications for next Meeting announced.
5. Specimens, Books, etc., presented.
6. Communications and Papers read.
7. Society adjourned.

CHAPTER III.

EXTRAORDINARY MEETINGS.

An Extraordinary Meeting of the Society may be called at any time, by authority of the Council, on the requisition of three or more Fellows.

CHAPTER IV.

ADMISSION OF MEMBERS.

SECTION I.—HONORARY FELLOWS.

1. The Honorary Fellows shall be limited to six British and twenty-five Foreign,—by British, being understood British subjects, whether resident in the British Islands or not.

2. The Council shall have the privilege of proposing Honorary Fellows,—the names of the gentlemen proposed being always stated in the Billet calling the Meeting at which they are to be balloted for. The election to be determined by a majority of at least two-thirds of the votes, provided fifteen Fellows are present and vote.

3. Any Fellow may submit to the Council the names of individuals whom he would wish proposed as Honorary Fellows; and should the Council decline to bring these forward, he may demand that they be balloted for.

4. Honorary Fellows shall be entitled to all the privileges of Ordinary Fellows, and shall receive copies of the *Transactions* free of charge.

SECTION II.—ORDINARY FELLOWS.

1. A candidate for admission into the Society, as an Ordinary Fellow, must present an application, with a recommendation annexed, signed by at least two Fellows. The application shall be read at the proper time during private business, and at the next Ordinary Meeting shall be determined by a majority of at least two-thirds of the votes, provided fifteen Fellows are present and vote.

2. Ordinary Fellows shall, on admission, sign the Laws, and pay the sum of Fifteen Shillings to the funds of the Society; and shall contribute Fifteen Shillings annually thereafter at the October Meeting. Ordinary Fellows are entitled to receive the *Transactions*, provided their subscriptions are paid.

3. Ordinary Fellows may at any time compound for their annual contributions by payment of Ten Guineas. They shall be entitled to receive the *Transactions* yearly as published.

4. Fellows who are not in arrear in their subscriptions, and in their payments for the *Transactions*, will receive copies of the latter, provided they apply for them within two years after publication. Fellows not resident in Edinburgh must apply for their copies, either personally or by an authorised agent, to the Secretary or Treasurer.

5. The Society shall from time to time adopt such measures regarding Fellows in arrears as shall be deemed necessary.

SECTION III.—CORRESPONDING MEMBERS.

Any person residing abroad may be balloted for as a Corresponding Member, on the recommendation of the Council.

SECTION IV.—ASSOCIATES.

The Society shall have power to elect by ballot, on the recommendation of the Council, Associates from those who may have acquired a claim on the Society by transmitting specimens or botanical communications. Associates have no vote in elections or in the transaction of the business of the Society, are not entitled to receive copies of the *Transactions*, and have no interest in the property of the Society.

SECTION V.—LADY MEMBERS.

Any Lady may become, on the recommendation of the Council, a Member for life on payment of a single contribution of Two Guineas, or may be elected and continue a Member on payment annually of a subscription of Ten Shillings; but Lady Members elected under this rule shall not be entitled to receive copies of the *Transactions*, shall have no voice in the management of the Society, nor any interest in the property thereof.

SECTION VI.—ORDINARY MEMBERS.

The Society shall have power to elect, on the recommendation of the Council, Ordinary Members, being ladies or gentlemen who, though not desiring to become Fellows, are interested in the objects of the Society. The name of any candidate proposed as an Ordinary Member shall be submitted to the Council on a formal application, signed by two Ordinary Fellows, to one of whom at least the candidate must be personally known, and, if approved, the election of such candidate will take place at next Ordinary Meeting. Ordinary Members shall have no vote in elections or in the transaction of the business of the Society, and they shall have no interest in its property. They shall pay the sum of Five Shillings on admission, and shall contribute Five Shillings annually thereafter, at the October meeting. They may also receive copies of the *Transactions* published subsequent to their admission, at prices to be fixed from time to time by the Council of the Society.

SECTION VII.—BALLOTING.

In the foregoing sections which relate to admission of members to the Society, the Council shall have power to recommend admission without ballot, unless such ballot is demanded by giving written notice to the Honorary Secretary two days before the date of proposed election. Any such ballot will be taken at the next Ordinary Meeting of the Society.

CHAPTER V.

OFFICE-BEARERS.

1. The Office-Bearers of the Society may be chosen from the Ordinary Fellows, and they shall consist of a President, four Vice-

Presidents, ten Councillors, an Honorary Secretary, an Assistant Secretary, a Foreign Secretary, and a Treasurer, who shall be elected annually at the Ordinary Meeting in October.

2. The Council shall annually prepare a list of Fellows whom they propose to nominate as Office-Bearers for the ensuing year. This list shall be printed and put into the hands of Fellows, along with the Billet of the October Meeting ; and Fellows shall vote by putting these lists into the ballot-box, with any alterations they may think proper to make. The lists shall not be signed. Every Fellow present at the Meeting is entitled to vote.

3. All the Office-Bearers may be re-elected, except the two senior Vice-Presidents and the three senior Councillors, who shall not be re-eligible to the same offices till after the interval of one year.

4. These Office-Bearers shall form the Council for the general direction of the affairs of the Society. Three to be a quorum.

5. The Council shall nominate annually an Auditor and an Artist, to be recommended to the Society.

6. The Council shall appoint annually three of their number, including the Honorary Secretary, who shall have power to co-opt additional referees, if necessary, to act as editors and superintend the printing of the *Transactions*.

7. The Council may at any time be called upon by the President, Vice-Presidents, or Secretaries, to meet with them for the transaction of private business.

8. The Council shall hold a Meeting for business on the second Monday before each General Meeting.

CHAPTER VI.

THE PRESIDENT AND VICE-PRESIDENTS.

It shall be the duty of the President and Vice-Presidents when in the chair, and of the Chairman in their absence, to conduct the business of the Society according to the order of the business laid down in Chapter II., Law 4, and to attend carefully to the enforcement of the Laws of the Society, and to signing the Minutes. The Chairman shall have a vote and a casting vote.

CHAPTER VII.

THE SECRETARIES.

1. The Honorary Secretary, with the aid of the Assistant Secretary, shall give intimation of all General and Committee Meetings, shall Minute their proceedings in Books to be kept for the purpose, and shall conduct all the Society's Correspondence in Britain. He shall also take charge of all Donations of Plants and Books, and shall see them deposited in the Herbarium and Library, in conformity with any arrangements made by the Society with Government.

2. The Foreign Secretary shall have charge of all the Foreign Correspondence.

Note.—Agreeably to an Act of the Town Council of the City of Edinburgh, dated January 8, 1839, the Professor of Botany in the University of Edinburgh is constituted Honorary Curator *ex officio*, with free access to the Society's Collection, whether a Member of the Society or not.

CHAPTER VIII.

THE TREASURER AND AUDITOR.

1. The Treasurer, subject to the inspection of the Council, shall receive and disburse all money belonging to the Society, collecting the money when due, and granting the necessary Receipts. His Accounts shall be audited annually by the Auditor appointed by the Society.

2. It shall be the duty of the Treasurer to place all money belonging to the Society in one of the Chartered Banks of this City, unless the same shall have been ordered by the Society to be otherwise invested ; and he shall never keep more than Ten Pounds of the Funds of the Society in his hands at a time. The Bank Account shall be kept in the name of the Society, and all drafts thereon shall be signed by the Treasurer.

3. The Treasurer shall, at the November Meeting, submit a certified Statement of the Receipts and Expenditure of the past year, with the Auditor's Report thereon.

CHAPTER IX.

VISITORS.

Each Fellow shall have the privilege of admitting one Visitor to the Ordinary Meetings of the Society at the close of the private business.

CHAPTER X.

ADDITIONAL LAW.

In the event of any Member acting in such a way as shall seem to the Fellows of the Society to be detrimental to its interests, the Council may recommend that the name of such Member be deleted from the roll. The recommendation shall be brought before the Society at its first Ordinary Meeting. It shall be finally decided at the immediately succeeding Meeting by ballot. If confirmed by a majority of two-thirds of the votes of at least fifteen Fellows, the name of such persons shall be deleted from the roll of Membership, and all his privileges connected with the Society shall be forfeited.

CHAPTER XI.

MAKING AND ALTERING LAWS.

Any motion for the alteration of existing Laws, or the enactment of new ones, shall lie over till the second Ordinary Meeting, and shall then be determined by a majority of at least two-thirds of the votes, provided fifteen Fellows are present and vote. The motion must be intimated to the Council, and shall be printed in the Billet calling the Meeting at which it is to be brought forward, and also in the Billet of the Meeting at which it is to be discussed.

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